ACOUSTIC IMAGING OF BRUISES

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ACOUSTIC IMAGING OF BRUISES

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SUMMARY

Ultrasound is a valuable tool to monitor wound healing. In this report, ultrasound is used to determine the features in the B-scans that correspond to a bruise. High frequency ultrasound scans show clear and distinct features that correspond to a laceration or a late stage pressure ulcer. This is because of the extensive damage and the rupture of the epidermis in both the cases. This study assumes significance because it is an effort to find such artifacts in the ultrasound scans of bruises caused by blunt forces where the epidermis remains intact. In this study, the structure of the skin was visualized using a 20 MHz ultrasound scanner. The B-scans were analyzed in MATLAB and LabVIEW. Investigation focused on the changes in the thickness and echogenicity of the top layer of the skin in the presence of a bruise. Skin thickness and echogenicity changes may result due to blood extravasations or edema. The characterization of the ultrasound device elicits optimal parameter settings to capture the best image. It also gives an idea about several external factors such as the force on the probe and the orientation of the probe which affects the B-scan and ways to overcome or minimize them. The best gain and depth settings were determined to maximize the signal to noise ratio and the resolution of the B-scans in the region of interest. The thickness values were calculated by averaging the distance between six sets of representative points on the boundary of the top layer. The skin echogenicity was calculated by performing a 2D FFT in a 16*32 pixel sliding window and weighting the higher frequency components. This ensured that the high frequency variations were emphasized more than the smooth, slower variations. The thickness and the echogenicity values are plotted against time to determine the trend in the variation of these parameters. We see an intraday and a daily fluctuation of skin

thickness and echogenicity albeit with no distinct trend on a day to day basis or between subjects. The results also give us a good estimation of the variation observable in these parameters in the event of an injury. The range of the ratio of bruised site to control site thickness was found to be between 0.8-1.4. The range of ratio of bruised site to control site echogenicity was found to be between 0.6 and 1.2.

A snapshot analysis is also performed, which describes qualitatively the structural changes in the B-scan of the bruise site compared to the control site. There are six different types of qualitative changes which can appear in the B-scan of a bruised site compared to the control. In the event of an injury, usually, more than one of these changes is manifested in the scan of a bruise.

High frequency ultrasound technology gives a high resolution image which can help us determine the presence of a bruise. Skin thickness and echogenicity vary considerably due to a number of physiological factors which can seldom be controlled. Therefore, these parameters can give conclusive evidence of a bruise only if the change between a bruised region and a control region is much greater than the daily, normal variations. Snapshot analysis can help detect a bruise or a deep tissue injury although it requires considerable experience with ultrasound B-scans of the skin. Further work involves the application of pattern recognition or face recognition algorithms to automate the detection.

CHAPTER 1

INTRODUCTION

Skin is the most injured organ in children and adults alike. It can occur due to accidental or non-accidental reasons and bruising is the most common form of cutaneous injury [27]. In cases of child or elder abuse, skin injuries are the most obvious signs [20]. The problem of differentiating abuse from accidental injuries in children or old people is one of significant magnitude. Another variation of damage to the skin is presented by the occurrence of pressure ulcers. Pressure ulcers mainly affect the elder population and people with mobility limitations. Pressure ulcers can lead to serious health complications like blood poisoning (septicemia), skin or bone infections. Pressure ulcers usually take a long time to heal and therefore it is important to prevent them. Prevention of pressure ulcers can significantly improve the quality of life for people especially those who are in specialized treatment facilities or those who require nursing care. The detection of early stages of pressure ulcers or bruises is difficult in people with darker skin tones due to the masking effect of melanin. Therefore, the development of a device which can aid in the detection and staging of either bruises or pressure ulcers in people with darker skin tones can help them prevent malignancies associated with skin injury.

1.1 Bruise

A bruise can be defined as “a hurt or injury to the body by a blunt or heavy instrument causing discoloration, but no laceration of the skin” [28]. A thorough analysis of bruising requires the examiner to determine the cause of the bruise, the time of occurrence of the bruise and more importantly to determine if the discoloration caused is

indeed a bruise. An impact, which causes the intravascular blood to have extravasate into the surrounding tissue, may or may not cause the bruise to appear immediately. In fact, the bruise may appear after several hours or may happen in some other place due to the flow of blood. The interpretation of bruises is difficult because of a large number of variables that affect the formation of a bruise. Bruising occurs easily in loose tissue such as that beneath the eyes as compared to strongly supported tissue such as that on the heel. The morphology of the surface of impact and the magnitude of the force affects the interpretation of the bruise. The appearance of the bruise depends on the skin coloration as well. So, the detection of bruises in dark skinned subjects creates extra challenges. There are numerous pathological conditions that cause the bruising to be disproportionately larger to the amount of force at impact. Conditions such as hypertension, cardiovascular degenerative changes and disorders of collagen are abnormalities that may predispose people to excessive bruising or bleeding [8].

Bruising, therefore, presents a lot of investigative challenges to clinicians who are asked to determine the cause and the origin of a bruise. They are often asked to distinguish bruises due to abuse as opposed to those due to accident. Previously, authors have done research to study and characterize a bruise using various approaches, from spectroscopy to ultrasound imaging [2, 11, and 19]. Some authors have devised a scoring system of bruises, which takes into consideration the dimensions of the bruise and the area in which it occurs [3]. This scoring system was then used to distinguish between abused and non-abused children. In spite of multifarious approaches taken towards the interpretation of bruises, there is no method that can characterize a bruise and track its cause definitively.

1.2 Pressure Ulcers

A pressure ulcer is a localized damage to the skin and subcutaneous tissue beneath due to the application of high pressure or friction. Erythema or skin redness is a precursor to pressure ulcers resulting from ischemia. It is usually seen after a prolonged exposure to pressure especially in areas over a bony prominence. This seriously affects people with limited mobility like the elderly and those who have spinal cord injuries. In 2001, the National Pressure Ulcer Advisory Panel reported prevalence levels in elderly people of 1.5 per 1000 for ages 65-74 and 8.1 per 1000 for ages 75 and older [25]. Prevalence of Pressure Ulcers in people with Spinal Cord Injury (SCI) is reported to be about 10-30% on their first annual examination.

Initial stage of pressure ulcer involves a persistent erythema that can progress to skin loss with tissue necrosis and damage to the bone or the muscle. The best way to stop the development of pressure ulcers is to detect erythema and take appropriate action to reduce the localized pressure or friction. However, erythema is masked by melanin and its detection becomes exceedingly difficult in subjects with darker skin tone. Because of the masking, a greater prevalence of Stage I pressure ulcers has been seen in white patients compared to black patients, but there is a greater prevalence of Stage III – IV ulcers in black patients [25].

Incipient Pressure Ulcers are a pocket of fluid which starts at the bony prominences in the body [29]. The location and the amount of fluids present can help clinicians in determining the cause of the Pressure Ulcer and also the stage it is in. Pressure Ulcers are divided into four different stages depending on the extent of tissue damage or its depth. The necrotic tissue can be the skin, underlying fat or muscle.

1.2.1 Stage I

A stage I pressure ulcer is an alteration of the normal skin which can be observable [30]. It can have indicators, compared to adjacent or contralateral site, such as different skin temperature, tissue consistency or sensation. It appears as constant redness in subjects with lighter skin tones. In subjects with darker skin tones it may appear to be persistent red, blue or purple.

1.2.2 Stage II

Stage II consists of partial excoriation of the skin. This tissue damage may extend beyond the epidermis into the dermal layers. The damage to the tissue is superficial and clinically looks like an abrasion or a blister.

1.2.3 Stage III

Stage III involves a full thickness skin loss with necrosis of the subcutaneous tissue. This damage may well extent to the muscle fascia. There may or may not be damage in the adjacent tissue. The pressure ulcer looks like a crater in the skin.

1.2.4 Stage IV

Stage IV consists of a full thickness skin loss at the site of the Pressure Ulcer. There will be complete tissue necrosis and damage to the bone or supporting structures.

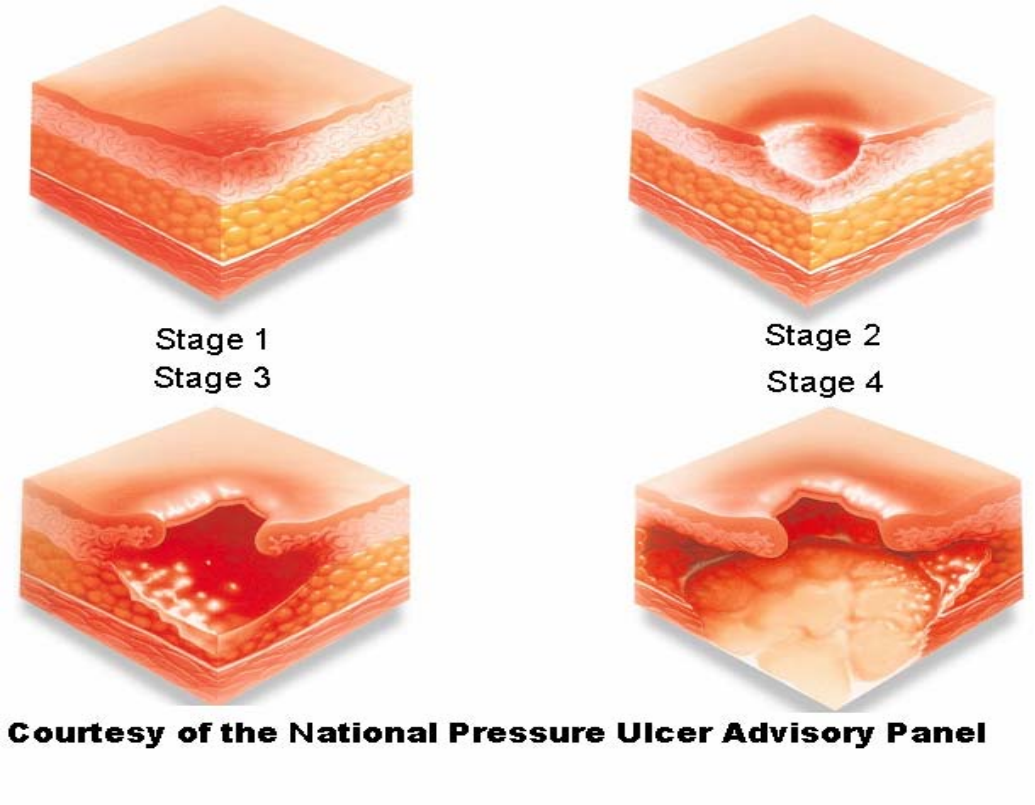


Figure 1: Stages of Pressure Ulcer

1.3 Ultrasound Imaging

The pooling of the extravasated blood in the skin due to a bruise causes a change in several mechanical properties of the skin. Ultrasound technology, hence, attempts to capture these changes and detect the presence of injury. If pooling of blood causes a change in the dermal layer thickness, it can be captured by using ultrasound waves at high frequencies in the order of 20 MHz. The presence of blood may also change the echogenicity of the bruised area which can be observed in the ultrasound scans.

Ultrasonic imaging modality helps the visualization of structures in the skin such as the epidermis, dermis, sweat glands, hair follicles, and stratified collagen layer in a non-invasive, reproducible and quantitative manner [10]. Ultrasound waves are generated by a

transducer at a particular frequency and that frequency decides the maximum depth and the resolution of the ultrasound image. Higher the frequency of the ultrasound modality, the depth of visualization in the skin becomes lower and resolution of the image increases. Ultrasound has been used in a plethora of dermal structure visualization such as the measurement of the skin thickness, observation of the effect of drugs, investigation of burn injuries and also the visualization and quantification of skin tumors [16, 31, 32]. Very little, however, has been written on the application of ultrasound imaging modality to evaluate the healing and development of wounds and bruises. Forester et al. [5] worked on the effect of the collagen content on the wound to the attenuation of the ultrasound signal. Gassmuller and Levy [6] used ultrasound to see the changes in echogenicity in a punch biopsy. They showed the re-epithelialisation phenomenon and that the dermal echogenicity structure was similar to the normal skin after healing. Hoffman et al [7] used ultrasound to measure the changes in the volume of the surgical wound to measure the effectiveness of healing of the wound to treatment. Rippon et al. [10] used ultrasound pulse echo technique to visualize dermal structures arising due to wounds and hence, track the healing of the wounds. Most of the work done is on pig skin since getting a biopsy of the human skin is not always feasible. Since wound healing is a complex process with multiple physiological and cellular components involved, the evaluation of healing requires a method which allows sequential, non-invasive, reproducible and quantitative measurements. Ultrasound imaging modality satisfies all the above criteria and hence is suitable to be used clinically to track the healing of a wound. Also, ultrasound signal is reflected by collagen consistently and thus can be used effectively to visualize protein. Collagen plays an important role in the healing of a wound and so

ultrasound can be used to quantify collagen in the wound area. This can help track the wound healing process. Therefore, ultrasound imaging can help in the detection of skin injury irrespective of the color of the skin. It can help us visualize the manifestations of a bruise or wound to a very high degree of accuracy. It has been used previously to track the healing of a wound and hence should be able to help us detect and track bruises.

1.4 Research Goals and Thesis Outline

Since both bruising and pressure ulcers are different variations of damage to the skin, the development of a device which can detect this damage irrespective of the numerous factors associated can be very helpful in a clinical setting. For this, it is critical to understand the stages of development of bruises and pressure ulcers along with the various factors which affect them.

Long term, this research aims to develop a handheld, clinically affordable device which can identify bruises and incipient pressure ulcers. This device can then be used by clinicians to scan the high risk areas and detect the presence of skin injuries. A combination of ultrasound and spectral imaging can help look for the features of the bruise and erythema. These two technologies are used to find the spectral signatures, texture and echogenicity features that can help us detect bruises and erythema. There is some evidence to show the relationship between ultrasound image features and spectral analysis. If that conclusion is confirmed by further data collection and analysis, this relationship can be used to enhance the feasibility of recognizing bruises and pressure ulcers practically.

This work focuses on the feasibility of using ultrasound imaging to detect features corresponding to bruises in the skin. Ultrasound helps us 'look' beneath the surface of the skin. Ultrasound imaging has been successfully used to stage bruises which were caused by sharp objects [11]. The breaking of the epidermis and the loss of tissues beneath the epidermis can be easily seen in the ultrasound B-scans. The challenge of this project is to detect and stage injuries caused by blunt objects where the epidermis is intact. Blunt injuries only cause damage to the tissue without any loss of the epidermis. The loss of epidermis is a significant clue in the detection of lacerations. Since the epidermis is intact in bruises caused by blunt trauma, other parameters will have to be considered to detect blunt injuries.

1.5 Hypothesis

Ultrasound B-scans of bruised subjects will be collected and analyzed to detect the features that corresponding to a bruise. Bruising leads to the pooling of blood or edema. The presence of pooled blood or edema creates a local change in the thickness of the top layer of the skin. The top layer of the skin consists of the epidermal and the dermal layer. In literature, the top layer thickness has been used as a parameter to detect the presence of certain pathological conditions or to determine the efficacy of a drug [31, 32]. The hypothesis is that a change in the thickness and the echogenicity of the top layer of the skin can help us detect the presence of a bruise. The change in thickness of the bruised site will be compared with the control site to see if the presence of a bruise manifests itself as a change in the top layer thickness. Similar experiments will be performed to determine the variation of the echogenicity of the top layer of the skin. A snapshot

analysis which compares a B-scan of the bruised site with that of a control site will help us elicit the qualitative changes in the scans of the bruised site. These categories can further be quantified using appropriate measures and hence, help detect the presence of a bruise. The hypotheses can be enumerated as below:

1. Ultrasound B-scans can be used to measure local changes in the top layer skin thickness over the course of bruise resolution.
2. Ultrasound B-scans can be used to measure top layer echogenicity changes in tissue over the course of bruise resolution.
3. Visual comparison of B-scans of bruised and normal sites can be used to identify the presence of a bruise.

CHAPTER 2

LITERATURE REVIEW

In the last 35 years on skin study, a lot has been written and understood about the histology and patho-physiology of the skin. Earlier work on bruises involved analyzing the color of the bruise to determine its age. The analysis was subjective and was not consistent between different researchers. Digital photographs were taken to analyze the color of a bruise. Alternative light sources have been used to enhance a physician's visual assessment of a bruise. This was done using Reflective Ultraviolet photography or infrared photography [20]. Ultraviolet rays have little penetration in the epidermal tissue where they are absorbed by various biochemical compounds present in the body. On the other hand, infrared rays have deeper penetration and have a theoretical possibility of detecting deep bruises or blood pools. These methods have some other limitations including the requirement of special lenses and settings, which do not make them a very convenient in a clinical setting. Vogeley et al. suggested the use of UV illumination using wood lamp, which makes it possible for sub-clinical bruises to be seen [20]. However, no mention of the depth of bruises that can be seen by illuminating with UV light is made.

Some other studies have concentrated on photographing the bruise and then comparing the color of the bruise with a color chart. Langlois and Gresham studied eighty- nine adults and obtained the photographs of bruises of known ages [8]. The color red appeared most of the time during the evolution of a bruise and hence, it could not be used as a good indicator of the age of the bruise. Blue and black could not be used to estimate time of aging because they were probably due to blood reflecting light at different depths in the skin. Yellow occurred in the later stages of the resolution of a

bruise but was also seen to occur as early as 24 hrs. The color yellow is associated with the presence of bilirubin and green is associated with the presence of biliverdin. Knowing the degradation process of hemoglobin the color green should appear before the appearance of yellow and since biliverdin is short lived, the color green may not be visible clearly to the naked eye. The authors stated that the color green could not be identified distinctly and looked more like a combination of yellow and blue. The conclusion from the study was that the presence of yellow color in the bruised region is significant and that such a bruise was at least 18 hours old. Stephenson and Bialas studied twenty-three children having accidental traumatic injuries [21]. Yellow color appeared in injuries that were more than a day old. They noted that green may occur as early as two days. Bruises of identical age and cause on the same person may not appear as the same color and do not change at the same rate. From all the above studies it is clear that aging of bruise is not a precise science and analysis of digital photographs of the bruise leaves several important issues unanswered. The bruise color analysis by other authors is as described in Table 1 [21].

Table 1: Schemes for the Aging of Bruises

Authors	Initial	1-3 days	1 week	6-10 days	2 weeks
Adelson	Red/blue	Blue/brown	Yellow/green		
Rentoule, Smith	Violet	Dark blue	Green	Yellow	Normal
Camps	Red	Purple, black	Green	Yellow	Normal
Polson, Gee	Red, black	Purple, black	Green		Yellow
Spitz, Fisher	Blue/red	Dark Purple	Green/yellow	Brown	Normal

The development of the portable high-frequency ultrasound equipment has made it easy for the ultrasound technology to be used in clinical studies for tracking the sub-

epidermal features of the skin. Monitoring the progress of wound healing, therefore, has been one of the most important applications. Most of the research has been directed towards finding the relationship between physical parameters of the skin and diseases, drugs, health and age. Skin thickness changes have been observed in various physiological and pathological conditions [1, 9, 15] and hence the hypothesis that a change in skin thickness will be observed in the presence of a bruise carries considerable weight.

2.1 Skin Histology and Basic Skin Study

D Black et al. [14] report a study in which the skin and the subcutaneous layers were measured to determine the effectiveness of pulsed ultrasound in the determination of subcutaneous fat thickness. Measurements were made using both ultrasound and Harpenden's skin calipers on skin covering the abdomen, scapula, triceps and front thigh on thirty nine subjects. The authors performed reproducibility analyses and method comparison analyses using a 10 MHz pulsed A-scan ultrasound system to measure the thickness. The reproducibility study was done with six people over three days. Every day three scans were taken on a particular body area. They concluded that the pulsed ultrasound technique is reliable and accurate. In the reproducibility study, the coefficient of variation increased with age, and the total coefficient of variation for the entire population of the study was 12.9% +/- 5.8%. However, the presence of septa in more obese subjects made it difficult to assess the thickness using ultrasound.

S. Shuster et al. [15] reported the measurement of skin thickness made using a xeroradiographic technique. The population of the study was large, including men and

women of all ages. The difference in the skin thickness between men and women was reported, and the relationship between skin thickness and age of the subject was found. Collagen content was determined by performing experiments using biopsies. Authors concluded that the skin thickness and collagen content decreased with age. The relationship between age and skin thickness for males and females is different. Male skin thickness decreased with an increase in the age of the subject. For females the skin thickness remained same until about 50 years and the decrease in skin thickness usually occurs after 60 yrs.

H. Alexander et al. [16] also used pulsed ultrasound to measure skin thickness and compare it with a radiographic method. Earlier methods to assess the skin thickness included Harpenden's skinfold calipers, radiographic technique by Black [33] which uses dangerous X-ray radiations and later Xenography. A 15 MHz pulsed ultrasound transducer was used to measure the thickness of the skin. A commercially available system with a resolution in soft tissue of approximately 0.05 mm was purchased and modified to be used in the skin thickness measurement. Water-skin, skin-fat ad fat-muscles interfaces could be easily seen in the reflecting echoes. They concluded that the ultrasonic measurements were reliable compared to measurements from x-rays and had a correlation coefficient r=0.99 and a rank correlation coefficient of r(s) = 0.91 was obtained making the correlation significant at the 95% confidence level.

C.Y. Tan et al. [24] made measurements similar to the Schuster, et al. In this work ultrasonic pulse-echo measurements were used to determine skin thickness of subjects. From those same sites, a 4 mm punch biopsy was taken to do the in vitro analysis. Skin thicknesses were in the order of 1 to 2.5 mm. On most parts of the body, the skin

thickness was in the 1.25 to 1.5 mm range. They showed the age thinning phenomenon and its difference in males and females. The study included a comparison of in-vitro (tissue removed and examined under a microscope) and in-vivo (tissue in-tact within the body) measurements. The results for the in-vitro measurements gave consistently larger values of skin thickness than either the xero-radiographic or ultrasonic measurements. This was attributed to the release of skin tension upon extraction. Skin thickness was found to increase linearly with age up to the age of 20 years and to decrease linearly with age subsequently. Pulsed ultrasound was used to do the in-vivo studies. The study also involved independent observation by two observers to do a reproducibility study. A strong correlation with r=0.88 between the measurements of two observers was seen. The intra observer coefficient of variation in their measurements was between 0 to 13.3 %. The authors comment that the research was motivated, in part, by the desire to evaluate a measurement method that did not use ionizing radiation.

Jo-Ann et al. [17] worked on Steller sea lions and adult harbor seals. An ultrasound imaging system with C60/5-2 MHz broadband, 60-mm head width transducer was used to measure the thickness of the skin including blubber thickness of the animals. These measurements were then compared with results from actual biopsies and were found to be 99.8% accurate. They could also see clear images of the epidermis, dermis and the blubber layer and could differentiate between phocid and otraiid blubber structure.

2.2 Wound Healing and Monitoring

Following injury, a normal mammalian skin has a predictable sequence of events which are designed to restore the normalcy of the tissue and prevent infection. First event

is the inflammation (early and late phase), granulation of the necrotic tissue and finally matrix formation and remodeling [36]. The inflammatory stage is also called the resting or the lag phase because very little appears to be going on beneath the skin. However, this is a very active phase and lasts several days. The second stage of granulation is also called the metabolic stage where the body produces collage to strengthen the wound. This stage lasts several weeks. The remodeling phase is the stage when the body models the scarred tissue and decides on the amount of scar which remains. Each of these events has characteristics which can be visualized by the ultrasound scanner.

The blood clot formation rapidly fills up the wounded area in the skin. This clot then acts as a scaffold for macrophages, fibroblasts, epithelial and endothelial cells. Blood vessels then sprout to supply nutrition to the new cells. With the progression of the healing phenomenon, granulation of the tissue can be observed. The granulation is immature in the beginning and then becomes mature with the deposition of type II and type III collagen. This granulation then fills up the entire wound area forming the scar tissue. The echogenic band on the surface of the wound is seen to be curving upwards sometimes because of hypergranulation [11]. The skin then overcompensates for it and curves downwards. This variation is what causes the top layer thickness of the bruised skin to vary with time until it attains a near constant value.

Ultrasound has been used in a plethora of dermal structure visualization such as the measurement of the skin thickness, observation of the effect of drugs, investigation of burn injuries and also the visualization and quantification of skin tumors. Very little, however, has been written on the application of ultrasound imaging modality to evaluate the healing and development of wounds and bruises. Forester et al. [5] worked on the

effect of the collagen content on the wound to the attenuation of the ultrasound signal. Gassmuller and Levy [6] use ultrasound to see the changes in echogenicity due to the presence of a bruise. Hoffman et al. [7] used ultrasound to measure the changes in the volume of the bruise to measure the effectiveness of healing of the bruise to treatment. Rippon et al. use ultrasound pulse echo technique to visualize dermal structures arising due to wounds and hence, track the healing of the wounds. Most of the work done is on pig skin since getting a biopsy of the human skin is not always feasible. Since wound healing is a complex process with multiple physiological and cellular components involved, the evaluation of healing requires a method which allows sequential, non-invasive, reproducible and quantitative measurements. Ultrasound imaging modality satisfies all the above criteria and hence is suitable to be used clinically to track the healing of a wound. Also, ultrasound signal is reflected by collagen consistently, it can be used effectively to visualize protein. Collagen plays an important role in the healing of a wound and so ultrasound can be used to quantify collagen in the wound area. This can help us track the wound healing process. The purpose of this study is to detect structural changes in the skin, such as acanthosis and edema which can be seen in the epidermal and the dermal layers. Since these structures are a precursor to skin ulcers or skin dehiscence, ultrasound may help us detect the patients who may be at risk to these pathological conditions.

M. Dyson et al. [2] compared two different types of technologies viz. photography and high resolution ultrasound scanning to assess the healing of a wound. The Longport EPISCAN centered at 20 MHz, was used in these measurements. Punctured wounds were induced on the subjects and tracked over a period of twenty one days. At regular intervals

the ultrasound and the digital photographs were taken to track the resolution of the bruise. Ultrasound scans showed a hypo-echoic region corresponding to the tissue damage in the bruise area. Even after the disappearance of the scab on the epidermis the ultrasound B-scans confirmed the presence of partial tissue damage. Authors concluded that the ultrasound technology scanning permits the quantitative measurements of several aspects of the bruise beneath the epidermis whereas photography can only capture the superficial aspects. Therefore, ultrasound scanning can be used to find the progress of the healing of the wound much better than digital photography.

Rippon M.G. et al. used a Dermascan® C, 20 MHz dermal scanner similar to the EPISCAN [10]. They provided results for depths up to 20mm. Echogenicity was used to determine wound area. Histological confirmation showed that blood clots formed in the wounds corresponded to the non-homogeneous echogenic areas within the wound. The authors, however, did not report a quantification of echogenicity. They used echogenicity as a means of defining regions within the acoustic images which were correlated with the histological data. The authors provided a thorough analysis of the physiology of the wounds and the healing process. They report that the ultrasound images confirmed that the early granulation tissue was echo poor and increased in echogenicity as the wound healed due to the increase in collagen content. In a separate confirmation, the fibrous granulation tissue was measured using hydroxyproline and correlated to echogenic regions of the ultrasound images. The authors confidently endorse the ability of the dermal scanner to visualize and quantify fibrous granulation tissue accumulation in wound tissue.

Rippon et al. [11] used ultrasonic pulsed echo imaging technique to characterize experimentally induced wounds in pigs. Pulsed ultrasound, Dermascan C 20 MHz transducer was used to view the tissue to a depth of 20 mm. The axial resolution was 0.05 mm and the lateral resolution was 0.3 mm. The author's interpretation of the sequence of events visually was consistent with the actual histological process of the healing of a wound. Stages such as granulation tissue formation, re-epithelialisation and wound contraction/fill can be visualized. Hence, ultrasound can be used to detect early signals of tissue breakdown prior to ulceration and to track the healing process of a wound.

It is seen that a growing number of results that point to the association of collagen content and echogenicity are seen. If the content of collagen or granulation tissue is altered by bruising, it can be hypothesized that echogenicity of a region in the skin can be used as a signaling characteristic for bruising. It should, furthermore, be a potential factor in the forensic analysis of bruises since echogenicity is lower in the wounded region and increases with time as the granulation tissue is restored. Authors conclude that Ultrasonography can be a useful, reliable, quantifiable technique for the assessment of wound healing.

2.3 Effect of Drugs on Skin Thickness

L Chen et al. reported a study in which high frequency diagnostic ultrasound was used to study the effects of hormone replacement therapy on skin thickness [1]. Skin thickness was used as an indicator of collagen content. The Longport EPISCAN with a frequency of 20 MHz was used in these measurements. The authors concluded that

hormone replacement therapy effectively increases the thickness of the skin of post-menopausal women.

Sator et al. studied the effects of three different hormone replacement therapies (HRT) on skin thickness [12]. A high frequency ultrasound system was used to measure the skin thickness on the inner side of the left upper arm of perimenopausal women with low oestradiol level. HRT resulted in the median value of skin thickness in all HRT groups increasing significantly by 0.15 mm after six months.

2.4 Relation Between Health and Skin Physiology

Since 5-8 % of the pregnancies are complicated by hypertension, N. G. Mirpuri et al. aimed to see the changes in abdominal skin thickness accompanying this medical condition of hypertension as compared to normal pregnancies [9]. The Longport EPISCAN, 20 MHz, was used in these measurements. Along with skin thickness, authors also analyzed changes in echogenicity by using fractal analysis. The fractal signatures of normal pregnancy were different from hypertensive cases. They proffer that if the abdominal skin of the patient does not get thinner as the pregnancy progresses then the patient may be hypertensive. Also, if these signals can be picked up early enough then potential high risk pregnancies may be identified.

A. Akesson et al. determined if high frequency ultrasound measurements can be used to improve skin characterization in patients with systemic sclerosis (SSc) by using skin thickness and echogenicity as characteristics [18]. Authors use a 20 MHz ultrasound transducer mounted in a water chamber. The measurements were performed at phalanx,

hand, forearm, and the chest of the patient. When compared with the control sites, the skin was thicker on all the four sites for patients with diffuse SSc but not with limited SSc after one year. However, after four years the skin thickness for diffuse SSc also decreased considerably for forearm and chest. Authors concluded by stating that high frequency ultrasound technology may be used as a non invasive tool to study SSc development.

Eisenbeiss et al. [4] investigated the change in dermal and subcutaneous tissue thickness to indicate water content changes in the skin. A B-scan device centered at 20 MHz was used in the measurements. The parameters were measured in twenty volunteers at baseline and successively at thirty minutes. They concluded that the skin thickness changes with a change in the water content in the body. This work directly relates to our work in terms of the types of measurement and the equipment used. The conclusions of this paper provide some of the best motivation for our methods. The authors indicate a change in dermal thickness and echogenicity with dermal water content. If the same is true for blood perfusion, then we should see a change in both of these quantities as well.

2.5 Mechanical Properties of the Skin

Over the past decade, several methods have been used to determine the elasticity of biological tissues [23]. These methods are collectively called elastographic techniques. The elasticity of the tissues is altered by the presence of a disease and hence a quantitative measurement of the mechanical properties of biological tissue is used by many researchers and doctors. Elastography therefore can be an effective diagnostic tool.

Even with a great need, there is very little reliable data on the properties of soft tissue. It is a common observation that the mechanical properties are non-linear, viscoelastic and have a strong dependence on the sample type. The variation of histology in the body tissues depends on the individual and hence standard properties of the tissues cannot be accurately defined.

When mechanical strain is applied to the biological tissue, the resulting stress is due to longitudinal and transverse stiffness [22]. The water content in the tissue affects its mechanical properties. The water content in the tissue is also a major factor which determines the thickness of the top layer of the skin.

M. Fink et al. measured the elasticity of the tissues with ultra high speed ultrasonic imaging [34]. The instrument provided the echogenicity of the tissues similar to a standard ultrasonic device but with a frame rate 200 time higher. The frequency of the device was centered around 60 Hz. It was used to detect the motion of the tissue in the body which was excited by low frequency shear waves. Fifteen patients who had a palpable breast lesion were chosen. The displacements of the tissues were found out and were mathematically inverted to find out the shear elasticity. Out of the fifteen patients examined six of them presented a clear and distinct tumor on the ultrasonic and elasticity images.

Pan et al. used high frequency ultrasound imaging to assess the elastic properties of the skin [35]. The experiments were performed in vitro. When the tissue undergoes a directional tension load the collagen fibers unwind and align along the direction of the stretching force. This structural rearrangement of the collagen fibers affects the ultrasound properties of the tissues. Samples of human and rabbit skin were used in the

study. An ultrasound biomicroscope with a center frequency of 28 MHz, developed in-house, was used in the study. It was seen that the Young's modulus of the human skin increased from 2.16 KPa at no strain to 26.95 KPa at 50% strain. For rabbit skin, the Young's modulus increased from 0.56 to 2.65 KPa for strain from 0 to 50 %. Authors also state that the elastic moduli of the epidermis, dermis and underlying tissue may differ considerably. Since this study used only intact skin, the results should be used as an average over the entire thickness of the skin.

The measurements of the mechanical properties are not consistent across subjects and have a wide range. The variation in the measurement of the mechanical properties and the skin thickness may be a manifestation of physiological factors like hydration, temperature, age. Hence, getting a consistent value for the mechanical properties across subjects may not very feasible.

2.6 Physical Meaning of the Data

Ultrasound imaging modality helps in the visualization of structures beneath the surface of the skin such as the epidermis, dermis, sweat glands, hair follicles, and stratified collagen layer in a non-invasive, reproducible and quantitative manner. Ultrasound waves are generated by a transducer at a particular frequency and that frequency decides the depth and the resolution of the ultrasound image. Higher the frequency lower the depth of visualization in the skin and higher the resolution. Looking at the ultrasound scans, the regions with high echogenicity (hyperechoic) are seen in lighter shades and the regions with low echogenicity (hypoechoic) regions are dark. The first echo obtained is from that of the latex cover of the ultrasound probe. The second

echo is from the gel-epidermal interface. Below the epidermis is the dermal layer which varies from 1.5 mm to 4 mm in the human body. The dermis can be divided into three regions, the papillary layer which contains fine collagen projections, the reticular layer which consists of coarse collagen fibers and the hypodermis which consists of loose adipose and connective tissue. Some parts of the reticular dermis may have subcutaneous fat invaginations where the coils of sweat glands may be found (26, 27). The ultrasound scans show hypoechoic regions interspersed with hyperechoic regions in the top layer of the dermis. Deeper down, a consistent hyperechoic region is noticeable which can be attributed to the fibers of collagen which are oriented parallel to the skin surface in the reticular layer. A hypoechoic region is then noticeable beneath the reticular layer reflections which can be attributed to the loose network of connective tissue and subcutaneous fat. Also, below the hypodermis can be seen the muscle fascia which is again echo-dense. However, at such a depth the attenuation of the signal from the layers above it should be given due consideration. Table 2 and Table 3 enumerate the components of normal and wounded skin along with their ultrasound characteristics [10].

Table 2: Ultrasound Characteristics of Normal Human/Porcine Skin

Skin Components		Ultrasound (20MHz) Characteristics
Epidermis	Stratum Corneum	Strong entry echo, dependent upon the thickness of the stratum corneum.
	Cellular Layer	Thin echolucent band, not usually possible to measure unless from an area that has a characteristically thick epidermis
Dermis	Papillary Dermis	Generally an echo-poor region, but interspersed with areas of high echogenicity.
	Reticular Dermis	An echo-rich region, the majority of the echogenicity originating from collagen fibers within the tissue. Collagen can be identified as bundles aligned paralle to the surface of the skin on the scans.
	Hypodermis	Generally an echo-poor region, but the fine collagen network that lies within the tissue can also be identified as bands of higher echogenicity.
Inclusions	Blood vessels Hair follicles Sebaceous glands	Generally all these inclusions appear to be echo-poor, although the blood vessel wall can be identified as having a higher echogenicity.
Muscle layer	Muscle tissue connective tissue	These areas can be identified as echo-rich bands, with a regular alignment of fibers within the bands themselves, although it is thought that the muscle tissue itself is echo-poor

Table 3: Ultrasound Characteristics of Components of Acute Experimental Wound Tissue

Wound components		Ultrasound characteristics of wound tissue
Blood clot	Fibrin/Fibrinogen	Intermediate echo-poor/echo-rich, depending on the densit of the clot
Granulation tissue Early (2-3 days)	Fibroblasts.macrophages Immature fibrous tissue Endothelial cells	Early stages generally echo-poor
Late (4-21 days)	Mature fibrous tissue (collagen)	Increase in echogenicity consistent with collagen accumulation.
Re-epithelialisation	Epithelial cells	Although the cells themselves were echolucent, a band of echogenic tissue could be identified at the surface of the tissue corresponding with re-epithelialisation. There may be other factors contributing to signal.
Scar tissue	Remodelled collagen	The disordered collagen fibres of a 3-month old scar are relatively echo-poor

This study looks at the change in top layer thickness and echogenicity in the presence of a bruise using high-frequency ultrasound. Ultrasound B-scans were captured using Longport EPISCAN system at 20 MHz. As mentioned earlier, our study assumes significance because most of the earlier studies have been with experimentally induced wounds and not bruises caused by blunt forces. Bruises caused by blunt forces change the problem of detection significantly. Firstly, the epithelial tissue is still intact and therefore the process of re-epithelialisation does not occur. The absence of the epithelial tissue is one of the clearest features used in the detection of a wound by ultrasound. Secondly, the

change in echogenicity in the dermal layers of the skin is not as remarkable as compared to punctured wounds. Therefore, a change in top layer thickness which accompanies the granulation in the bruise due to collagen formation is important to detect the presence of a bruise and its subsequent healing.

Very little has been done to analyze the features of the bruise cause by blunt forces or trauma by using high-frequency ultrasound B-scans. There is no mention of the evolution of the physical properties of the skin with the evolution of the bruise from infancy to complete healing.

CHAPTER 3

METHODS

3.1 Characterization of the Longport EPISCAN

3.1.1 Description of the Measurement Device

The system (Longport EPISCAN) consists of a portable computer and has a probe attached to one of its ports. The computer has a Windows 98 operating system. The EPISCAN uses an ultrasound transducer that operates at a frequency of 20 MHz. This makes it suitable to scan to a depth of about 3 cm, which gives a very high resolution (65 microns) in soft tissue. The probe is filled with water and is covered with latex finger-cot to prevent any spill. Gel is applied to the surface of the finger-cot and the probe is then placed on the subject. The transducer rides on a motorized drive screw and has a travel of 1.5 cm. Depending on the depth of the scan settings, the B-scans appear on the screen. These images can be stored in the computer in various formats. The depth of scan can be chosen among various options present in the software. The entire process is non-invasive and painless. The entire procedure takes about a minute once the probe is ready.

Figure 2: Longport EPISCAN

B-scans of the bruised and the control sites were taken to investigate if the thickness and the echogenicity of the top layer could be good indicators to distinguish bruised sites from un-bruised or control sites. In this report the combined thickness of the epidermal and the dermal layer will be called the ‘top layer’ thickness.

3.1.2 Calibration of the Device using Phantoms

This section describes the experiments that were undertaken in order to test and characterize the EPISCAN. One of the first problems faced was the variation in parameters required for effective image acquisition for subjects with different tissue characteristics and even different body parts which results in a change of skin strata thickness and depth. Experiments were performed with various phantom materials, which approximate the human tissue, to characterize the parameters associated with the ultrasound scans. The ELASTACK molding materials came in three different grades i.e.

easy pour, intermediate grade, tough grade. These phantom materials were of different modulus and elasticity and hence, gave a good variation to test most of our hypothesis and parameter choices. Introduction of bubbles or water bodies in the phantom gave a crude imitation of the bruises inside the skin surface. Considering the fact that these experiments were only for the optimization of parameters and not to draw a parallel to the bruises in a tissue, the experimental results could be applied with a high degree of confidence to the clinical settings. As a result, we have been able to find optimal parameter ranges pertaining to different situations.

3.1.2.1 Depth of Focus

Variation of amplitude of a boundary with depth settings: A phantom 2.5 cm X 5.0 cm X 10 cm was fabricated using a Plaster of Paris mold. ELASTACK was melted in a wok at 375 degrees Fahrenheit and was poured into the mold. It was allowed to set overnight. The gain was set to zero. The probe was clamped and immersed in water using an adhoc setup built in-house. The phantom was placed beneath the probe and the distance between the surface of the transducer and the top phantom surface was varied. Nine scans of the phantom were obtained with change in the depth of motion. These scans were then analyzed to determine the amplitude of the top phantom surface as seen in the ultrasound image. This amplitude was then plotted with its corresponding depth. The result obtained is shown in Figure 6.

3.1.2.2 Gain Settings

Determination of optimal gain value: Determination of the gain curve that will help us to determine an optimal range for the gain settings. The probe was placed on the phantom and the gain was varied from 0 to 100 %. From the scans obtained, we determined the RMS value of the region in between the upper and the lower surfaces of the membrane, which theoretically, should not contain any signal. The RMS value of the pixels in that region will give us a good indication of the noise associated with increase in gain. The images obtained were then filtered and the RMS value of the pixel in the region of interest was plotted against its corresponding gain. The results are as shown in Figure 7.

3.1.3 Optimization of Measurement Conditions

Data acquisition through the EPISCAN ultrasound device requires a carefully controlled setup which helps minimize the effects of extraneous factors on the data. The orientations of the probe, the pressure applied to the subjects via the probe were some of the factors that were considered. Experiments were performed to determine the changes which occur in the data because of these external factors.

3.1.3.1 Force applied on the Probe

B-Scans were taken to determine the effect of varying probe force on the ultrasound images. This was important to find out the variation in results between different people taking the data and also the intra-operator reliability. This variation was found by a very

simple experiment. Ultrasound images were taken with different forces applied on the probe. The force applied on the probe was not measured. The change in the force on the probe was within the range of the force usually applied on the subjects by the operator. Specifically, the top layer thickness measurements were performed to see if the thickness varied with the amount of pressure applied.

3.1.3.2 Orientation of the probe

B-scans were collected to determine the effect of probe orientation on the top layer thickness of the images. This was important to clear doubts about the orientation needed to get the best ultrasound images. Questions about the orientation of the probe relative to the body surface became crucial especially in areas which were hard to access. First a region on the body was chosen and ultrasound image was taken with the medial-lateral orientation of the probe. Next, ultrasound image was taken at the exact spot on the body but with a proximal-distal orientation. These images were then analyzed for skin thickness to find out the variation due to the orientation of the probe.

3.2 Thickness Measurements

The thickness of the top-layer of the skin was measured by selecting six pairs of points on the boundary of the top layer. The boundary was determined visually and not by using any computer program or software. These points are shown as black boxes in Figure 3. Using the six sets of points on the boundary the vertical distance between the corresponding upper and lower boundary points gave the thicknesses of the top layer at those points. The thickness of the top layer was the average thickness at those six points.

The calculation program was written in MATLAB and the six points were picked manually.

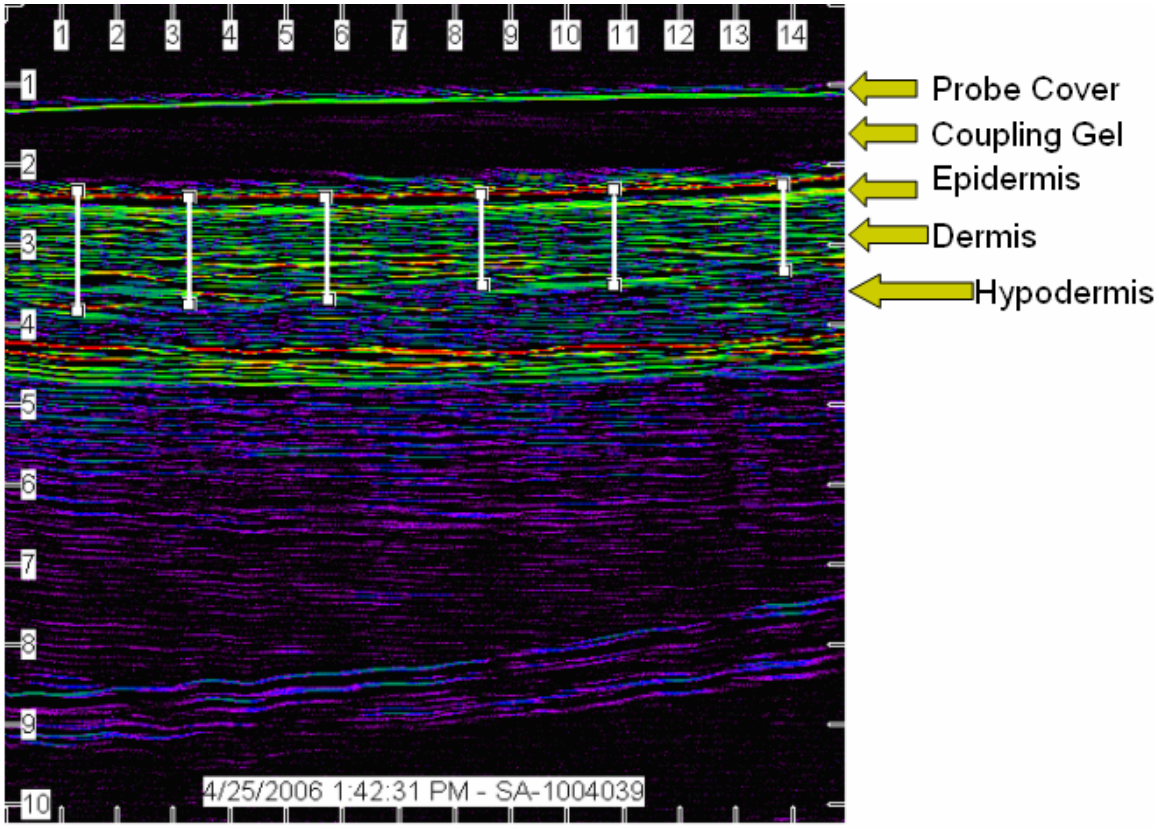


Figure 3: Method for Measuring Top Layer Thickness

3.2.1 Variation in Thickness of Normal Skin

3.2.1.1 Variation over Five Minute Intervals

In order to find out the variability of the normal skin thickness over a period of time, controlled experiments were performed on a single non-bruised site on a subject over a period of 12 days on the dorsal forearm. To ensure that the same site was scanned every

time a mole on the subject's forearm was taken as the site of measurement. Each day twelve scans of that site were taken at intervals of 5 minutes. The results would show the intraday variation in a subject and also the bigger time scale daily variability. An estimate of the standard error of the mean in the daily variations was obtained from these measurements.

3.2.1.2 Variation over Two Minutes

Similar experiments were performed to look at the normal skin variation over two minute intervals. The only difference in this protocol was to take alternative scans of the normal and the contralateral sites. The site of the scan was still the same spot as earlier on the subject's forearm. The data obtained was used to determine the variation on contralateral sites over two minute intervals.

3.2.1.3 Variation within Eight Seconds

To investigate the top layer variation over a shorter time scale, a modified approach was used. A unique feature in the Longport EPISCAN was that the motor driving the transducer could be stopped and now instead of a B-scan of a particular region, we would have a series of A-scans of one single point on the body. The EPISCAN can take about 512 A-scans in a second. The EPISCAN also has the capability of storing the last eight scans taken by the transducer. A series of A-scans on a single point on the body was taken and stored. The same location used during the five minute experiment was selected; this location was a mole on the subject's forearm. The method for determining the change in the top layer thickness was unlike the method described previously. This is because it

was difficult to determine the exact boundaries of the top layer. This limitation was overcome by taking a signal which can be used as a representation of the upper or the lower boundaries. The processing of the data was done in MATLAB where representative signals of the skin boundaries were picked manually. These representative signals for the upper and the lower boundary are shown by yellow lines in Figure 4. These signals are then cross correlated across the top layer boundaries to find the displacements in the upper and the lower boundary. These displacements were then combined to find the change in the thickness of the top layer. This signal was then used to correlate across the entire set of A-scans to determine the change in skin thickness.

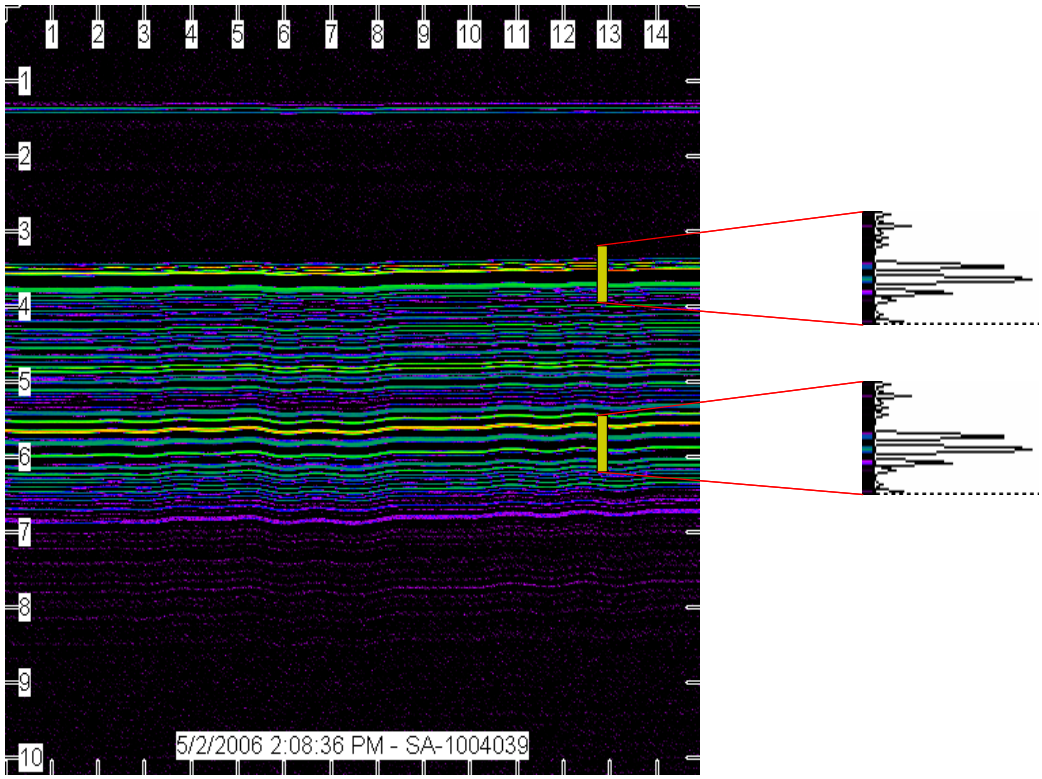


Figure 4: A-Scans of single point on the body

3.3 Echogenicity Measurements

Echogenicity of a region in a B-scan is a measure of the amount of acoustic energy reflected by the corresponding region in the skin. Echogenicity of the top layer of the bruise is measured by performing the spatio-temporal spectral processing at every point in the image. At each point on the image, a two dimensional Fast Fourier Transform (FFT) is performed in a sliding window of size 16 pixels * 32 pixels. Echogenicity was used as a measure to determine the texture in the ultrasound image. Since the interest was to find out the texture of the image at each point, the smooth variations had to be de-emphasized. To do this, spectral components were weighted to de-emphasize the lower frequency components. The weights were determined by trial and error. The set of weights which gave clear manifestations of different regions in the B-scans was chosen. These weights were then used to linearly combine the magnitude of the spectral components and plotted in the new image. Similar calculations were done at every single point on the image by moving the window to that point. Even though the echogenicity values were calculated for the entire image, the region of interest for our measurements was the top layer of the skin. Figure 5 shows the schematic diagram for the measurement technique described. The region of interest for the echogenicity measurements is the top layer and is shown approximately by the yellow box in the figure.

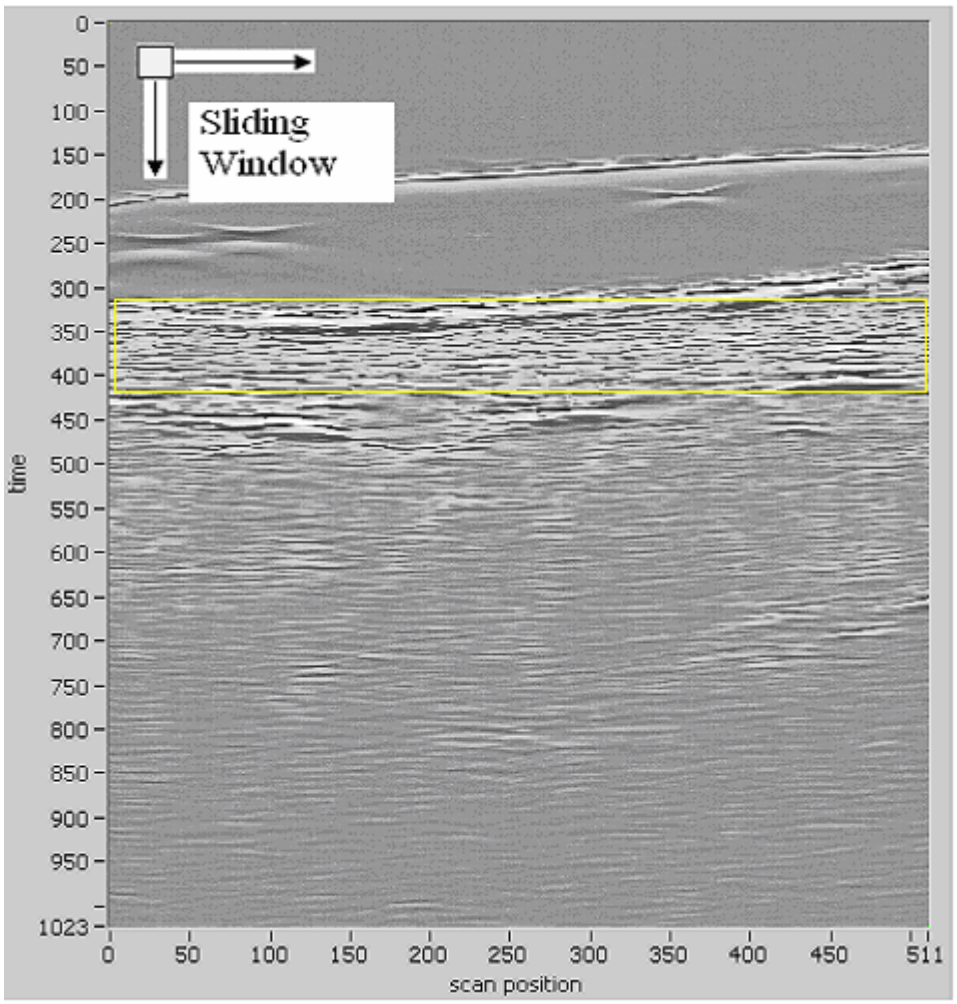


Figure 5: Method for Measuring Echogenicity

The program to do the spatio-temporal spectral processing of the image was written in LABVIEW by a team member. In the manual mode, after the weighted spectral component magnitude was obtained, the upper and the lower boundaries of the top layer were demarcated by five pairs of points on the image. A third order polynomial was fit to approximate the entire boundary. After fitting a polynomial to the upper and the lower boundary the echogenicity measurements were made on the images.

In the automatic mode a reliable indicator of the upper boundary and the lower boundary was needed. It was noted that there was always a low frequency-low magnitude component in all the images which corresponded to the entry echo at the epidermal-gel

boundary. This was used as a reliable signature for determining the epidermal-gel boundary in all the images. The lower boundary was then estimated by finding the point at which the echogenicity exhibited the sharpest change i.e. by noting the point of the highest slope. After finding the top and the lower boundaries the echogenicity was calculated for all the pixels between them. This value of the echogenicity was then normalized with the top layer thickness and hence the echogenicity values used in the analysis is a normalized measurement and is very robust to slight changes in boundary shape.

3.3.1 Echogenicity Measurement using RMS Values

The calculations for echogenicity were done by calculating the Fourier transforms and taking the weighted sum of certain frequencies. RMS value over the top layer could also have been used as a measure of echogenicity. To see how echogenicity measurements using the above described method is better than the RMS measurements echogenicity was calculated using both the methods and statistical and qualitative differences in the two methods were found.

Coefficient of Variation (COV) is a measure of variation normalized by the mean of the data set. This allows a comparison of different datasets. Top layer echogenicity was calculated using FFT method and the RMS method. Repeated measurements were performed using the RMS and the FFT methods. The repeated measurements were used to calculate the COV of the two datasets.

3.3.2 Variation in Echogenicity of Normal Skin

Echogenicity was calculated using the same datasets obtained for the skin thickness variation studies. Normal skin scanned every five minutes for 60 minutes over a twelve day period was used. An estimate of the SEM for echogenicity was obtained from the results.

CHAPTER 4

RESULTS AND ANALYSIS

4.1 Characterization of the Longport EPISCAN

Characterization of the ultrasound device was performed to determine the optimal set of parameters which would give the best possible B-scan. The study aimed to determine the depth of focus of the transducer, gain settings, probe orientation.

4.1.1 Depth of Focus

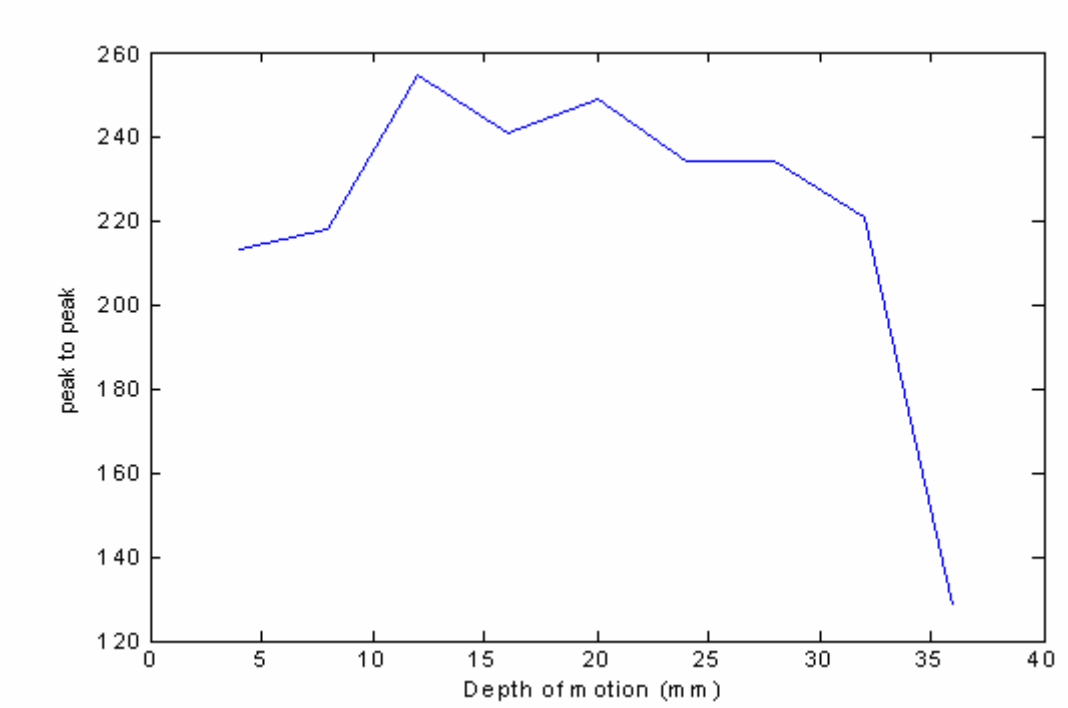


Figure 6: Plot of Peak to Peak Amplitude vs. Depth of motion

The acceptable peak to peak values were considered greater than or equal to 230. From Figure 6 it is clear that best readings are obtained with in the depth of focus, which lies in between 12 to 28 mm from the surface of the transducer.

4.1.2 Gain Settings

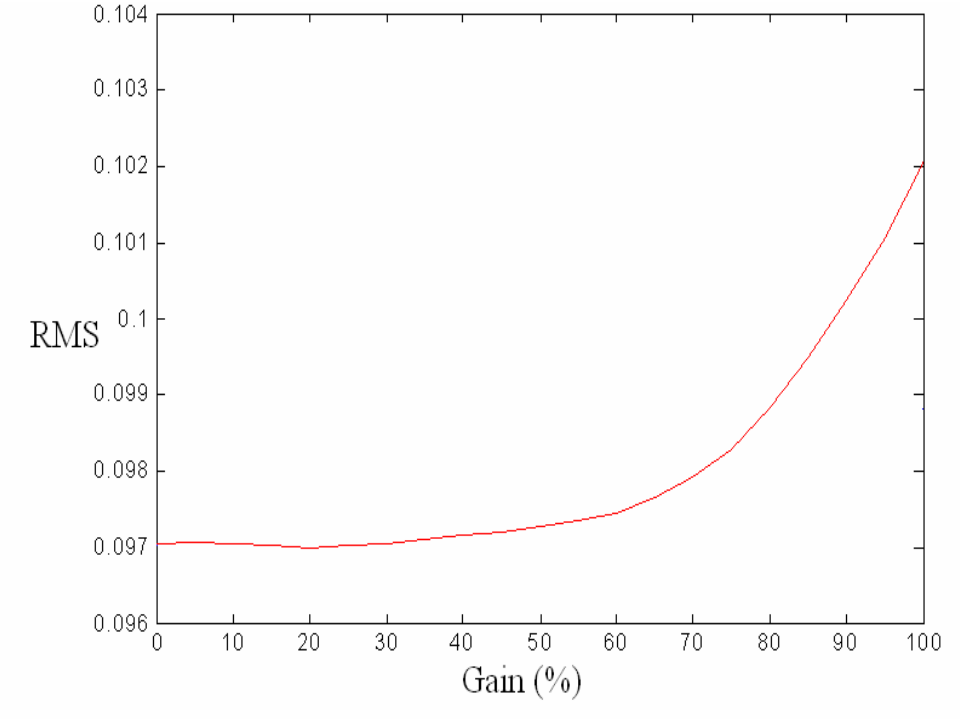


Figure 7: Variation of RMS value of the image with Gain

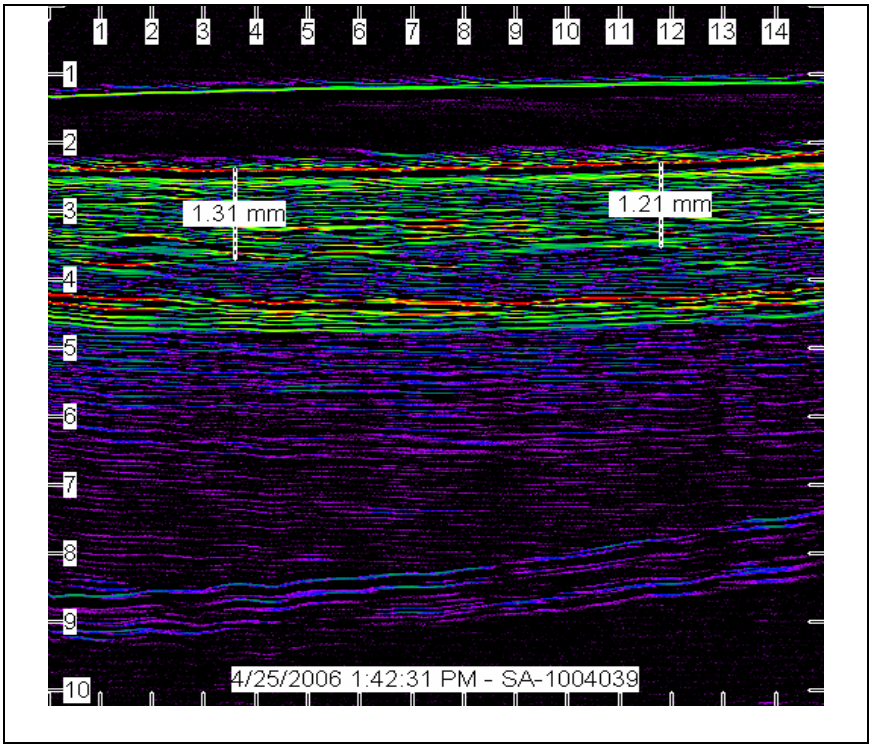
Figure 7 shows the change in the RMS value with the increase in gain settings. From the figure we see that the RMS value is relatively constant between 0 and 45 %. After 45% the signal and the noise increase simultaneously. Hence, it was decided to set the gain value at 40 % in our measurements.

4.2 Optimization of Measurement Conditions

4.2.1 Force Applied on the Probe

To find out whether a change in the force applied on the probe effect the thickness and echogenicity measurements different forces, qualitatively, were applied. Care was

taken not to deform the tissue and keep the force uniform across the area of the probe. The mean thickness and echogenicity in the first case as shown in Figure 8 were 1.20 mm and 1241. The mean thickness and echogenicity in the second case with a different force on the same body site were 1.24 mm and 1250. The thickness and echogenicity were calculated with the methods describer in the previous section. From the results we see that there is not a significant change in the skin thickness and echogenicity with a change in the force on the probe.



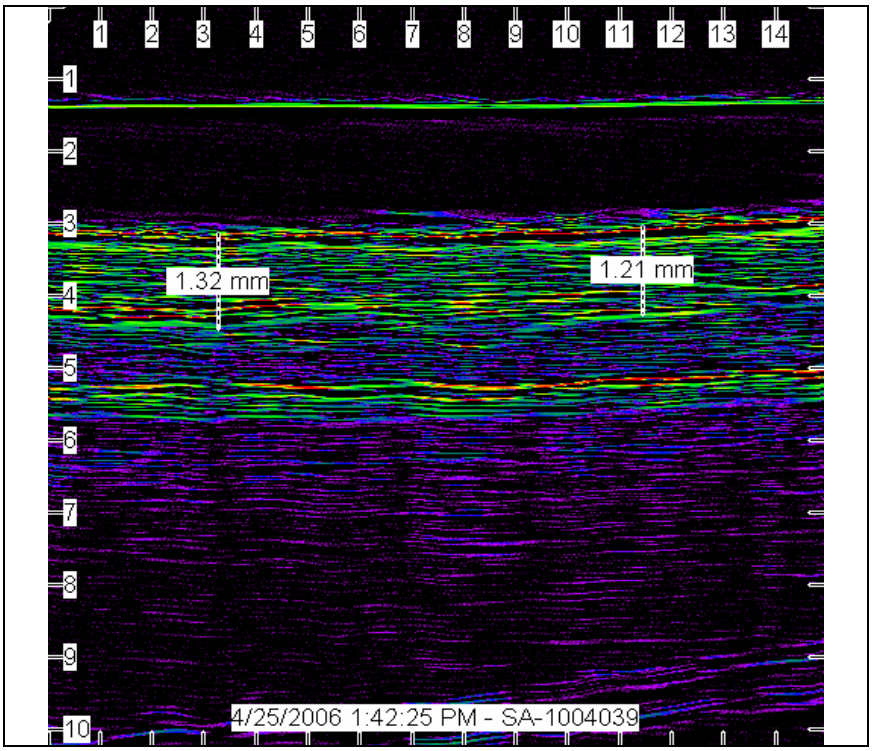


Figure 8: Comparison of B-scan with different forces on the Same Region

4.2.2 Orientation of the Probe

To find out the difference in the thickness and the echogenicity of the top layer of the skin with change in orientation of the probe, scans were taken on a particular body site with different orientation i.e. medial-lateral vs. proximal-distal. The mean thickness and echogenicity calculated using techniques described in the methods section, in the first case as shown in Figure 9 were 1.20 mm and 1241. The mean thickness and echogenicity in the second case in Figure 10 with a different orientation on the same body site were 1.18 mm and 1279. From the results we see that there is not a significant change in the skin thickness and echogenicity with a change in the probe orientation.

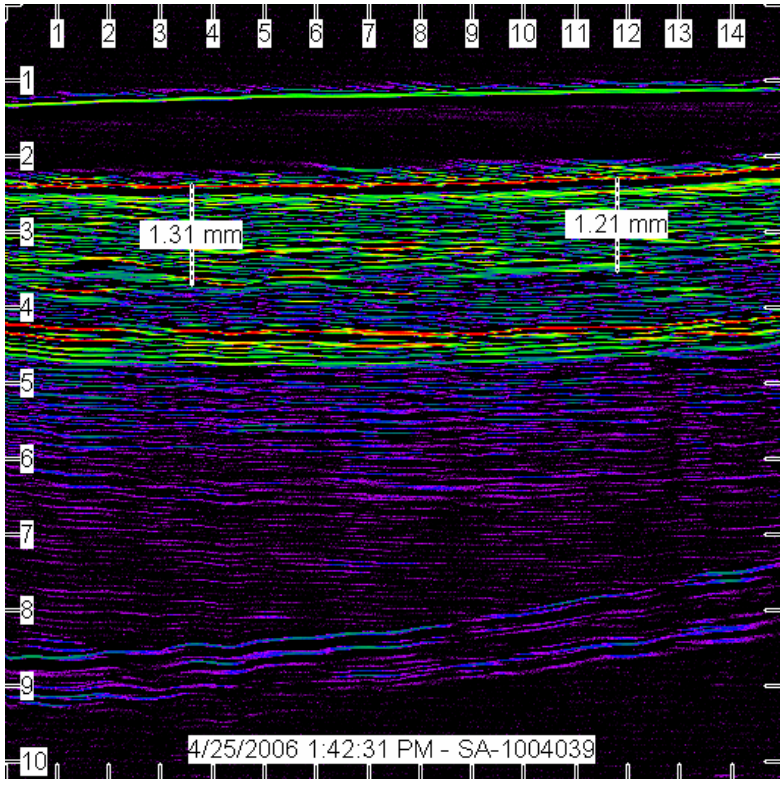


Figure 9: B-scan with Medial-Lateral Probe Orientation

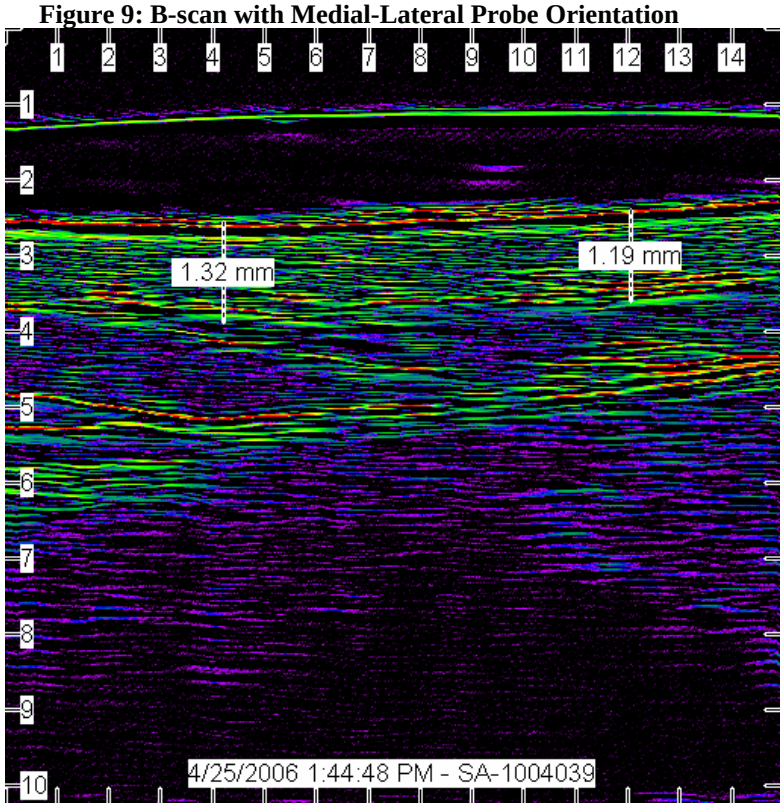


Figure 10: B-scan with Proximal-Distal Probe Orientation

4.3 Normal Skin Repeated Measures

In order to find the variability of the normal skin thickness over a period of time, controlled experiments were performed on a single off bruise i.e. control site on a subject over a period of 12 days on the dorsal forearm. To ensure that the same site was scanned every time a mole on the subject's forearm was taken as the site of measurement.

From Figure 11, Figure 12 and Figure 13 it is clear that there are daily variations in the skin thickness measurements. From the entire sample collected, it was determined that the Standard Error of the Mean (SEM) for top-layer thickness was 0.02 mm. The confidence limits for 95% confidence level for the normal skin thickness measurements are 1.317 ± 0.0392 at this site. Figure 12 only shows the variation over seven days for clarity purposes.

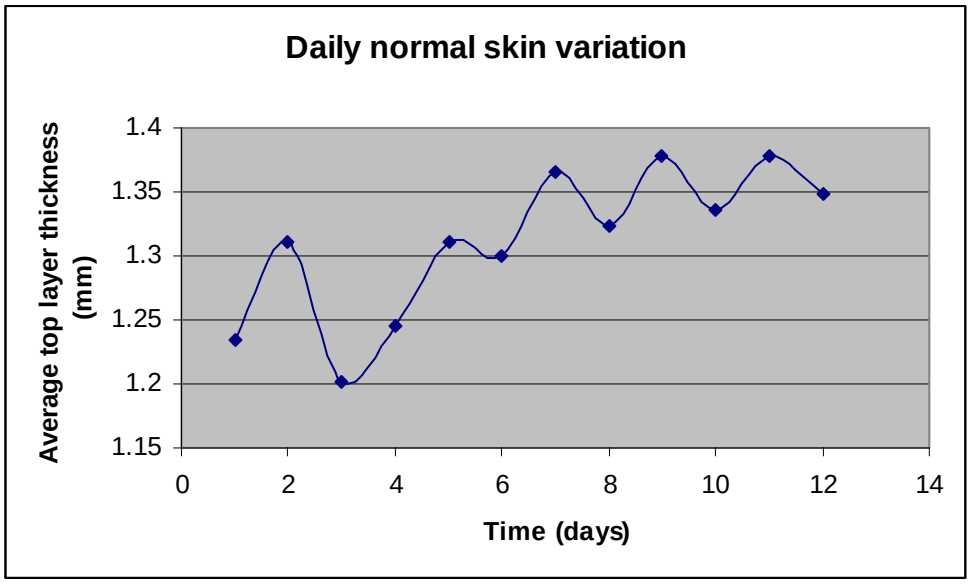


Figure 11: Daily Normal Skin Thickness Variation

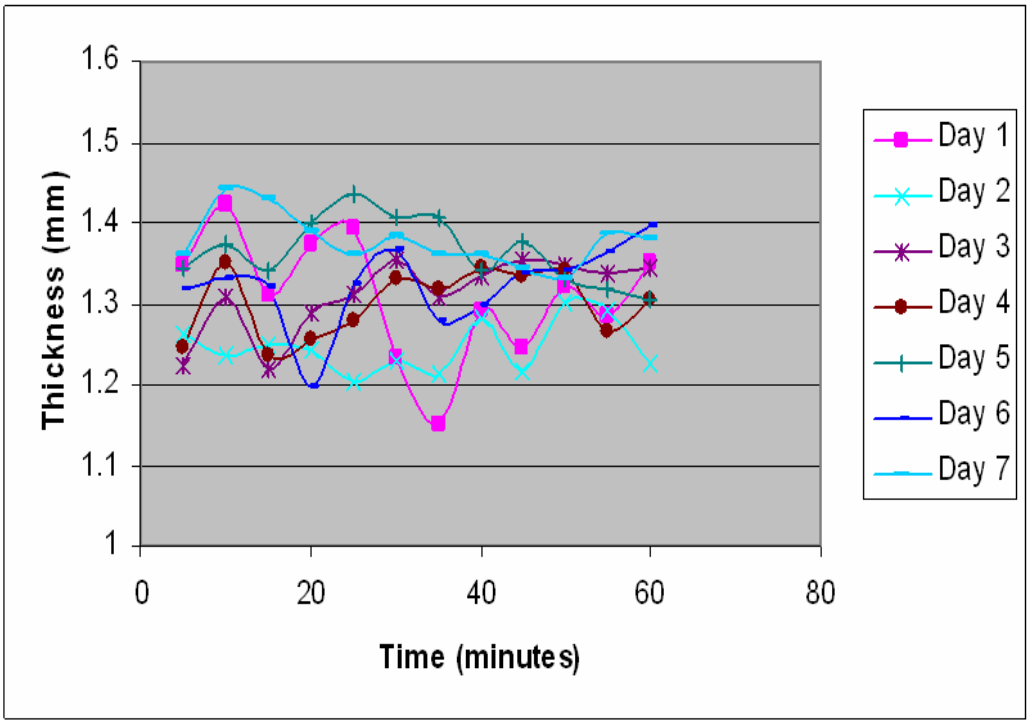


Figure 12: Intraday Normal Skin Thickness Variation over Five Minute Intervals

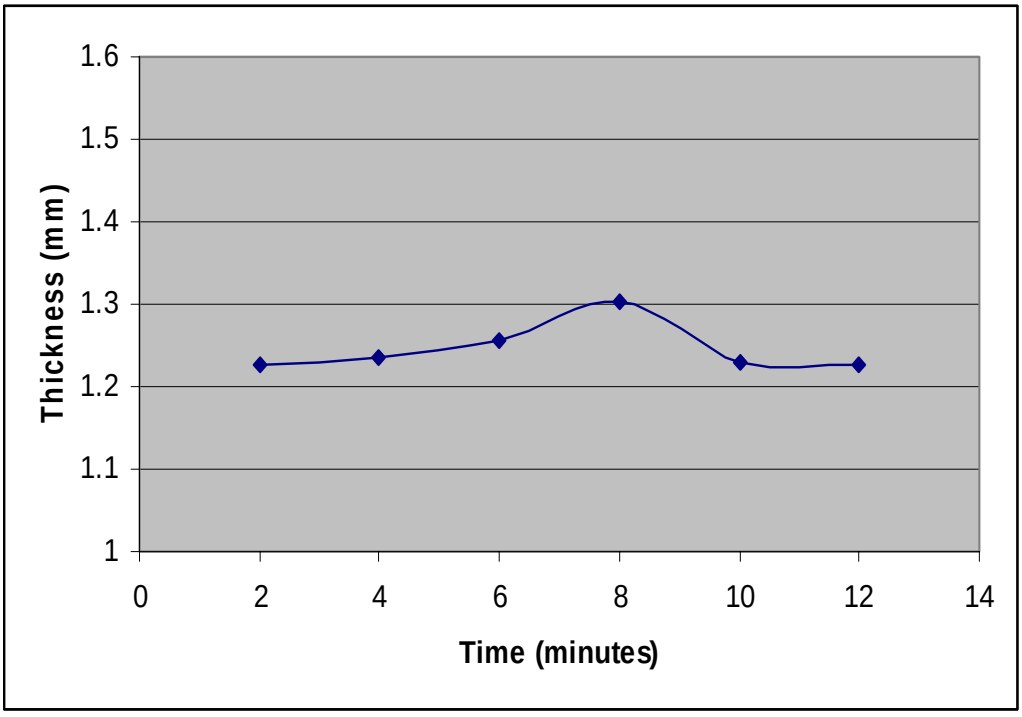


Figure 13: Intraday Normal Skin Thickness Variation over Two Minute Intervals

Table 4: Daily Thickness Variation over Five Minute Intervals

Measurement day	Mean thickness (mm)	Std deviation	SEM	Coefficient of Variation
1	1.23	0.048	0.018	0.038
2	1.31	0.076	0.021	0.058
3	1.20	0.044	0.015	0.037
4	1.24	0.032	0.009	0.026
5	1.31	0.047	0.013	0.036
6	1.30	0.042	0.012	0.032
7	1.36	0.040	0.011	0.029
8	1.32	0.051	0.014	0.038
9	1.38	0.031	0.009	0.023
10	1.33	0.048	0.014	0.036
11	1.38	0.063	0.018	0.046
12	1.35	0.063	0.018	0.047
Overall	1.32	0.070	0.020	0.053

Figure 14 shows the variation in skin thickness within 7 seconds. 512*7 A-scans of a particular point on the body was captured for about 7 seconds. From the figure we can still see considerable differences in skin thickness within such a short time frame. Figure 15 shows the variation of a point on a piece of plexiglass, which should be constant. The variations are very small and give an estimate of the error induced in our measurements due to the operator or the machine.

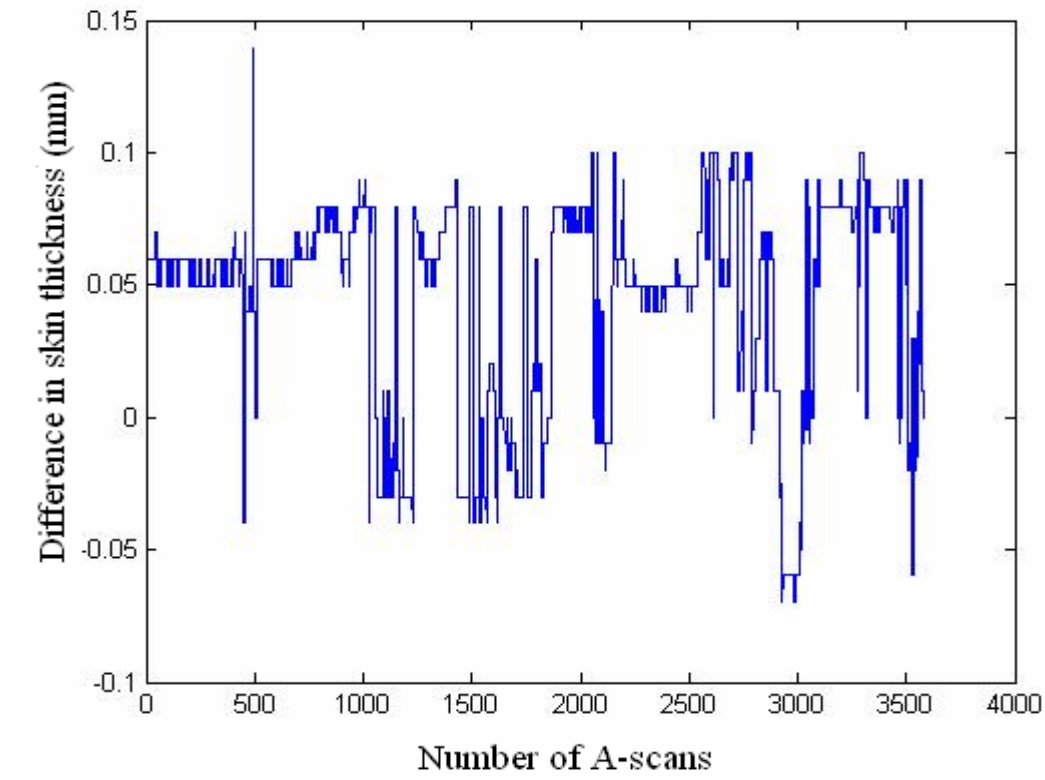


Figure 14: Variation in Skin Thickness within 7 seconds

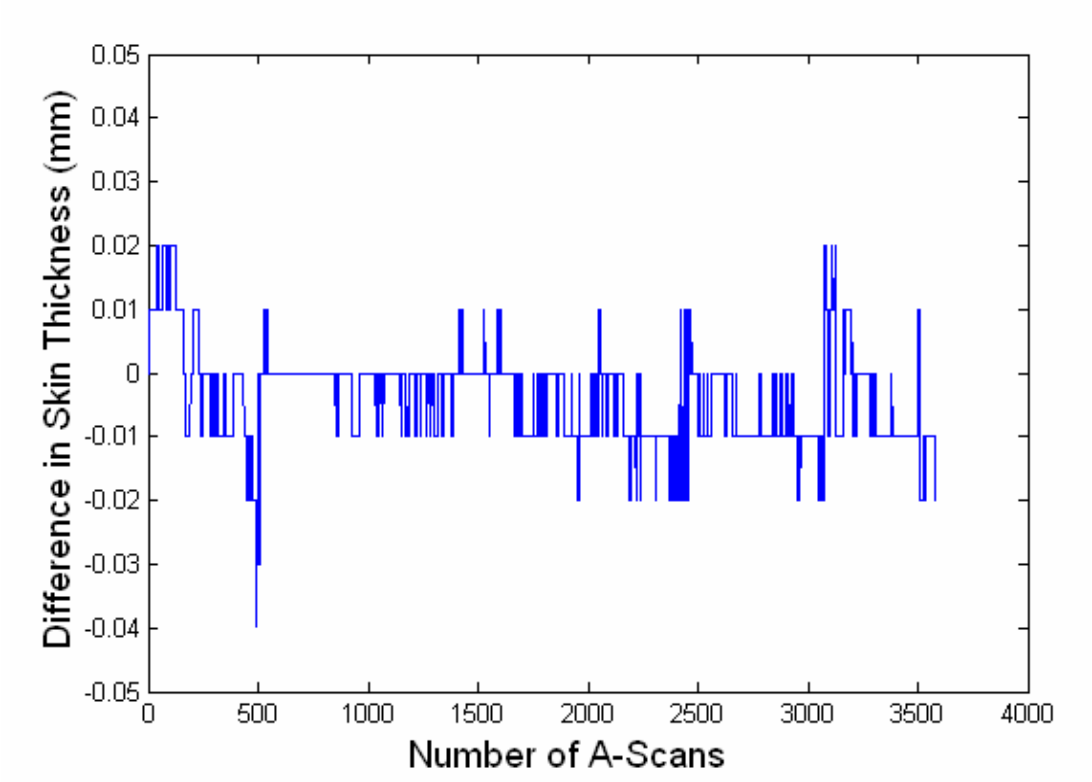


Figure 15: Thickness Variation on a Piece of Plexiglass

Figure 16 shows a comparison between contralateral sites. This helps us determine the variation in contralateral site thicknesses with time.

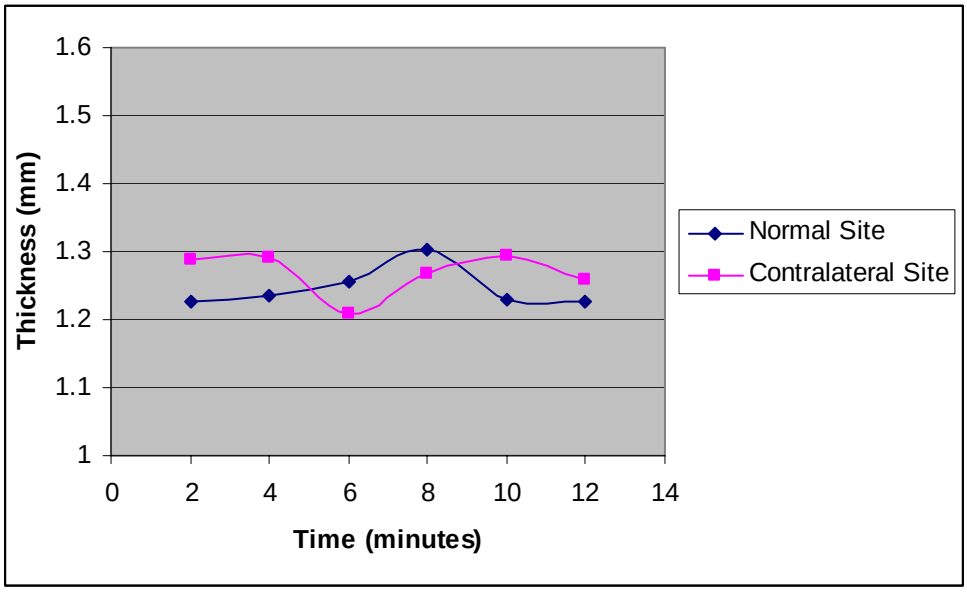


Figure 16: Comparison of Contralateral Site thickness

A comparison of echogenicity using the FFT and the RMS method was done to see which of them was better in the detection of the boundaries of the top layer. The COV for echogenicity measurements using the FFT method was 0.003 and the COV for echogenicity measurements using RMS method was 0.01. Since inter-operator reliability is a concern in our analysis, the method with the lesser COV is preferable.

Figure 17 shows that the variation in echogenicity along the depth of the image is smooth and hence different layers in the B-scan can be found out easily. Figure 18 shows that the echogenicity variation is abrupt all along the depth of the B-scan. Finding out the actual boundaries of the top layer using the RMS method is therefore not very practical.

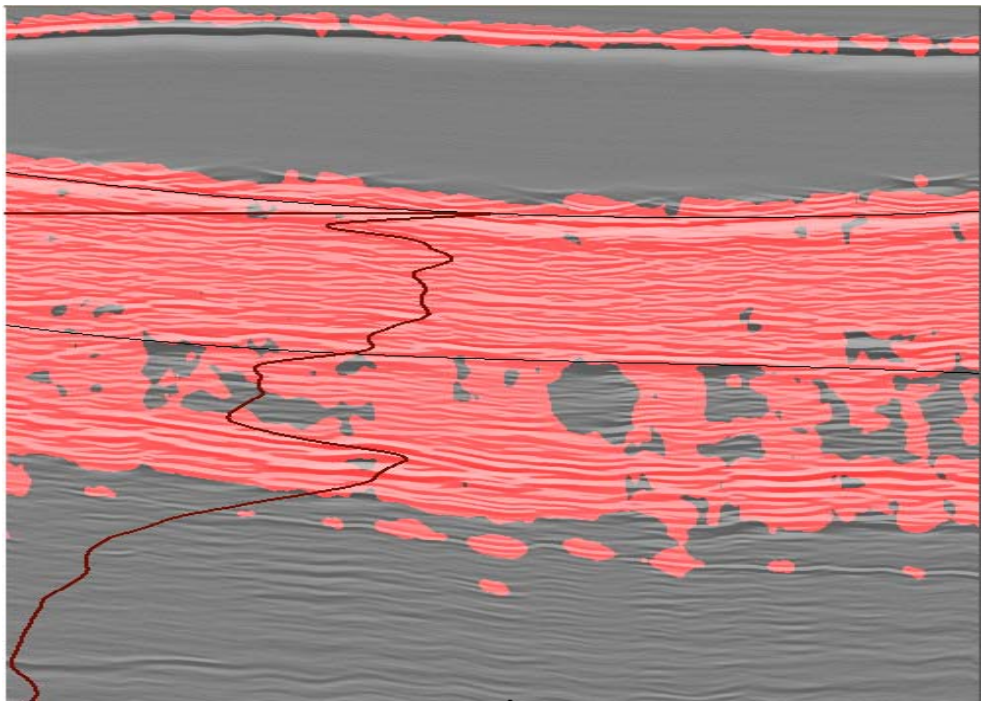


Figure 17: Echogenicity Variation using FFT Method

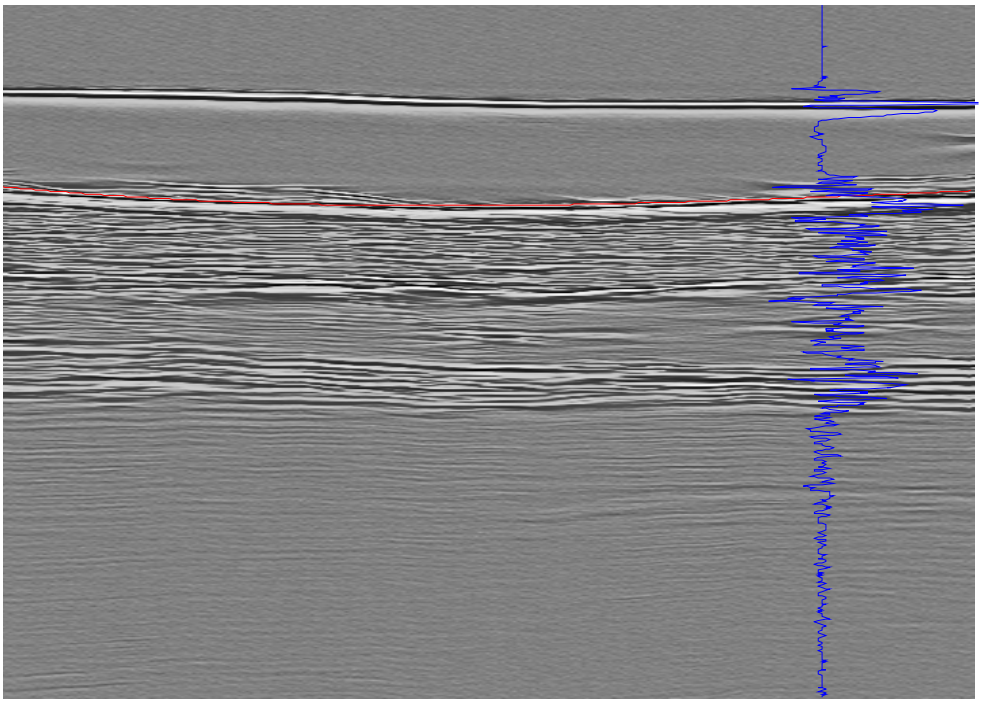


Figure 18: Echogenicity Variation using RMS Method

Echogenicity measurements were also plotted with time similar to the thickness measurements. It can be seen from Figure 19 and Figure 20 that there are daily variations in the echogenicity of the top layer as well. It is important that the gel region be completely bubble free since the presence of bubbles can affect the echogenicity measurements significantly. The analysis of echogenicity data should also include normalization of the data to account for these variations. Figure 20 only shows the variation over seven days for clarity purposes.

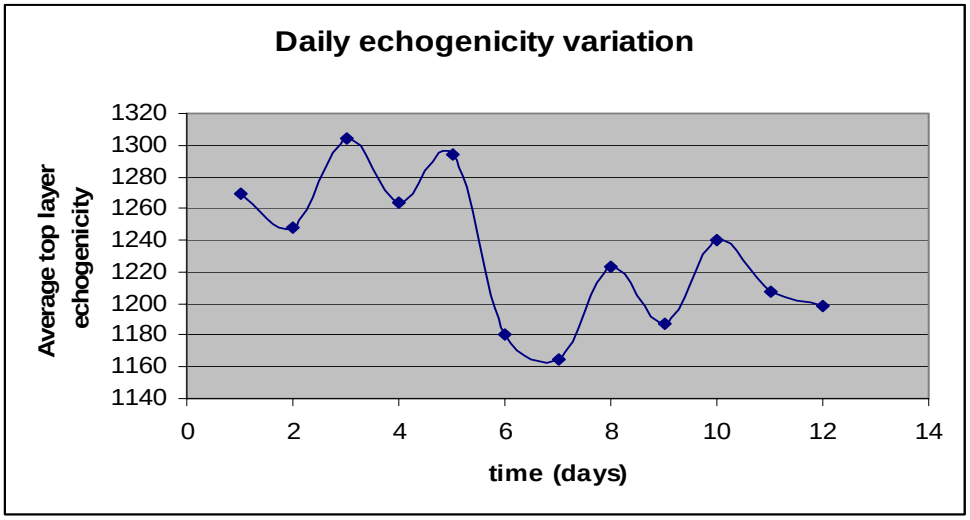


Figure 19: Daily Echogenicity Variation

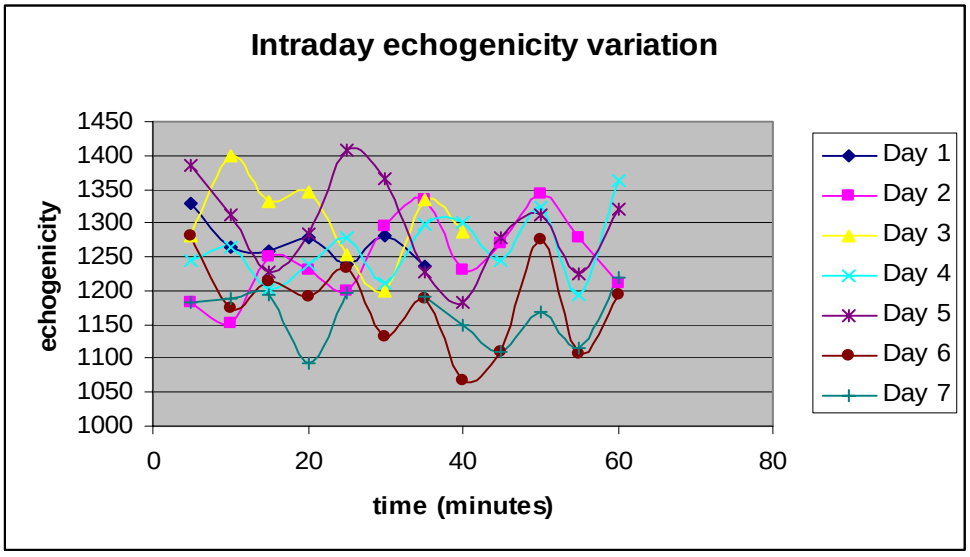


Figure 20: Intraday Echogenicity Variation over Five Minute Intervals

Table 5: Daily Echogenicity Variation

Measurement day	Mean Echogenicity	Std deviation	SEM	Coefficient of Variation
1	1269.429	31.18	11.78	0.024
2	1248.417	58.45	16.87	0.047
3	1304	61.50	21.74	0.047
4	1263.917	51.21	14.78	0.040
5	1294.417	69.73	20.13	0.053
6	1180.25	66.99	19.33	0.056
7	1164.455	41.65	12.02	0.035
8	1222.818	65.76	18.98	0.054
9	1187	56.63	16.34	0.048
10	1240.667	83.69	24.16	0.067
11	1207.364	90.35	26.08	0.075
12	1198.333	55.59	16.04	0.046
Overall	1230.211	74.82	21.60	0.060

The SEM for the echogenicity variation was found to be 21.6. The confidence limits for 95% confidence level for the normal skin thickness measurements are 1230.211 ± 42.336 for this site.

4.4 Variation of On-bruise versus Off-bruise Thickness with Time

In order to look at the variation of the on-bruise thickness with time, B-scans of the bruised area of several subjects were collected. The average thickness of the top layer of the skin was measured using MATLAB. The echogenicity measurements were done in LabVIEW. The results below show the variation of the top layer thickness and echogenicity over the period of bruising. Some bruises healed with in a matter of weeks and some took about two and a half months. In order to compensate for the day to day variations induced by skin water content, blood pressure and other physiological variables the ratio of the on bruise measurements to the off bruise measurements are

plotted. The error bars in all the graphs below are the SEM values for that particular parameter which was determine earlier. Table 6 lists the details of the subjects in our measurements.

Table 6: Subject Details

Subject ID	Subject age	Subject gender	Bruise location	bruise age at 1st measurement	number of measurements	Span of measurements (days)
B0316B	46 yrs	Male	Bicep	4 days	8	10
B1111A	36 yrs	Female	Right posterior medial calf	1 day	6	21
B1103B	NA	NA	Medial anterior thigh	3 days	8	46
B0929A	42 yrs	Female	dorsal lateral right forearm	1 day	4	7
B0930A	36 yrs	Female	Anterior shin	Less than 1 day	4	8
B060120A	86 yrs	Male	Left forearm	~2 weeks	5	14
B1028A	42 yrs	Female	Right lateral proximal thigh	1 day	11	21

4.4.1 Subject B0316A

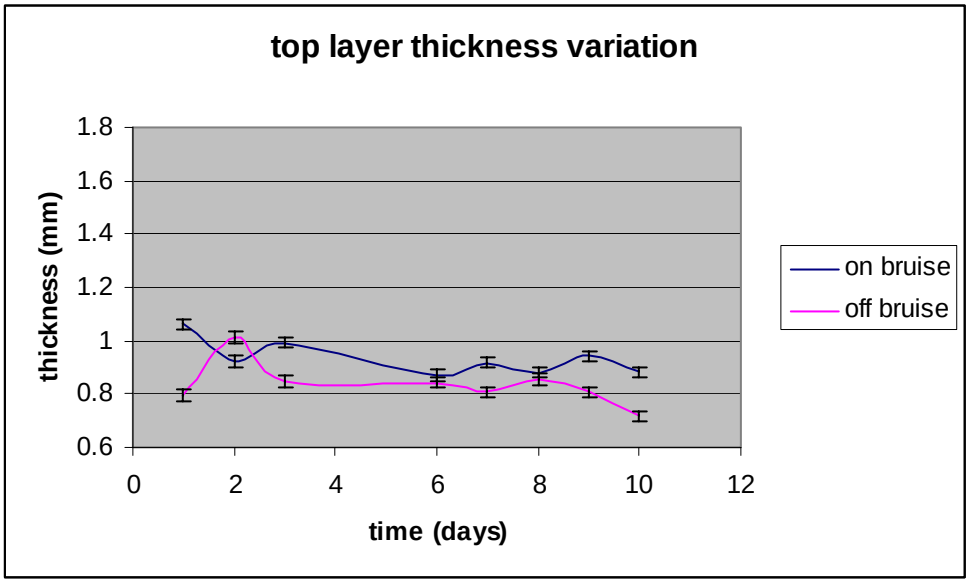


Figure 21: Top Layer Thickness Variation

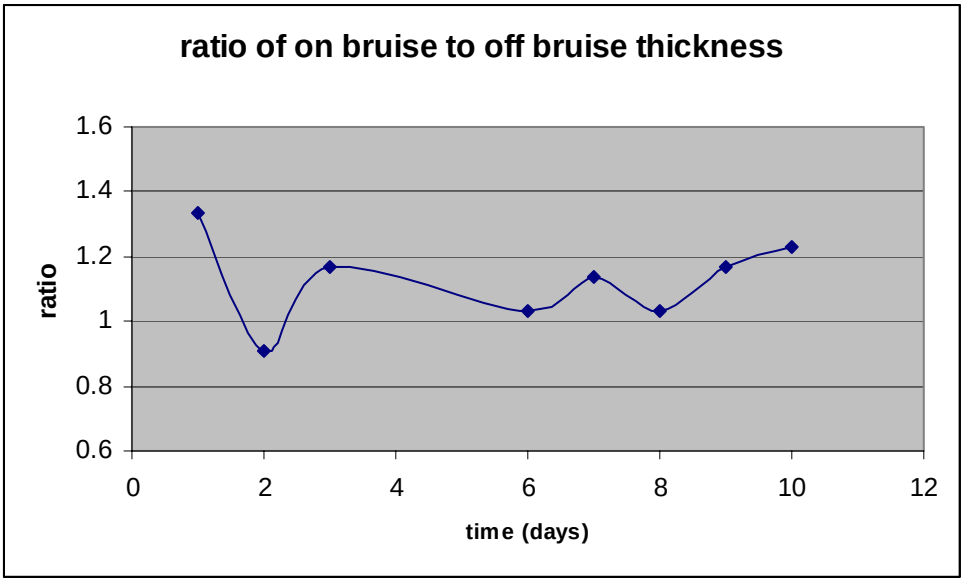


Figure 22: Ratio of On Bruise to Off Bruise Thickness

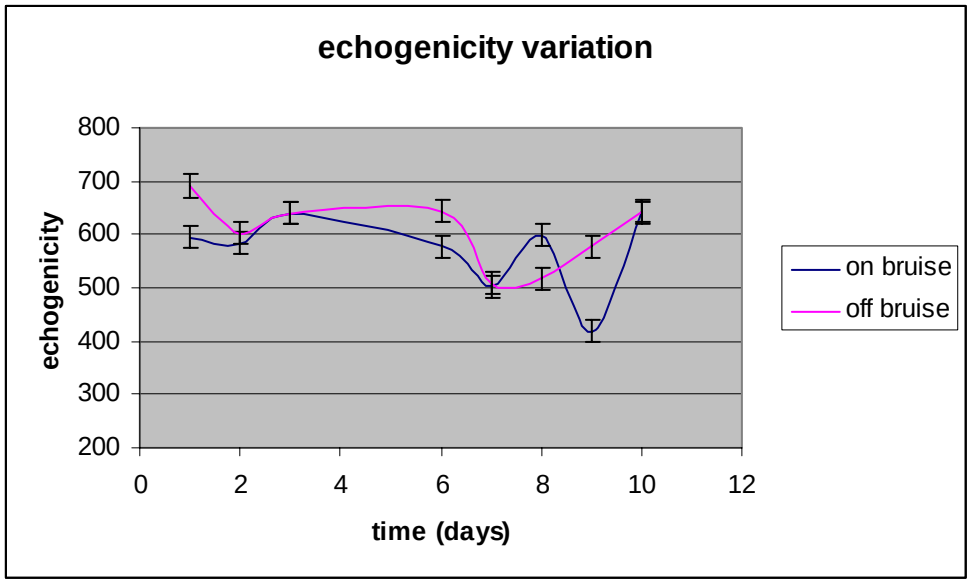


Figure 23: Echogenicity Variation

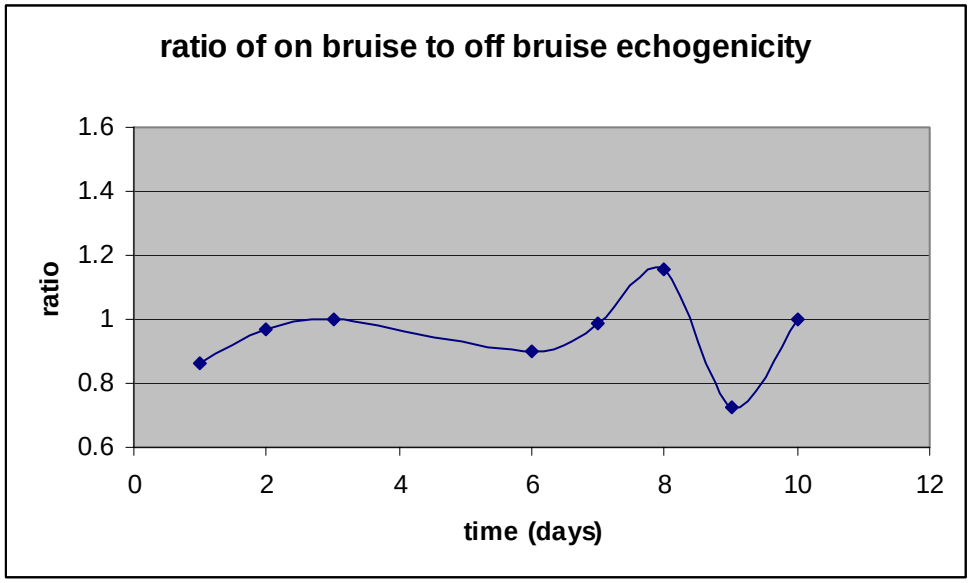


Figure 24: Ratio of On Bruise to Off Bruise Echogenicity

4.4.2 Subject B1111A

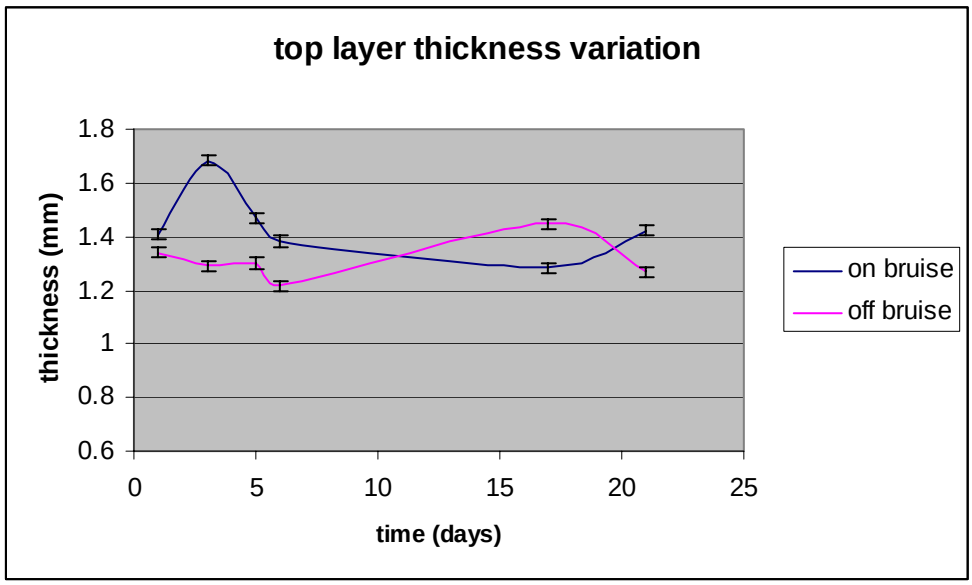


Figure 25: Top Layer Thickness Variation

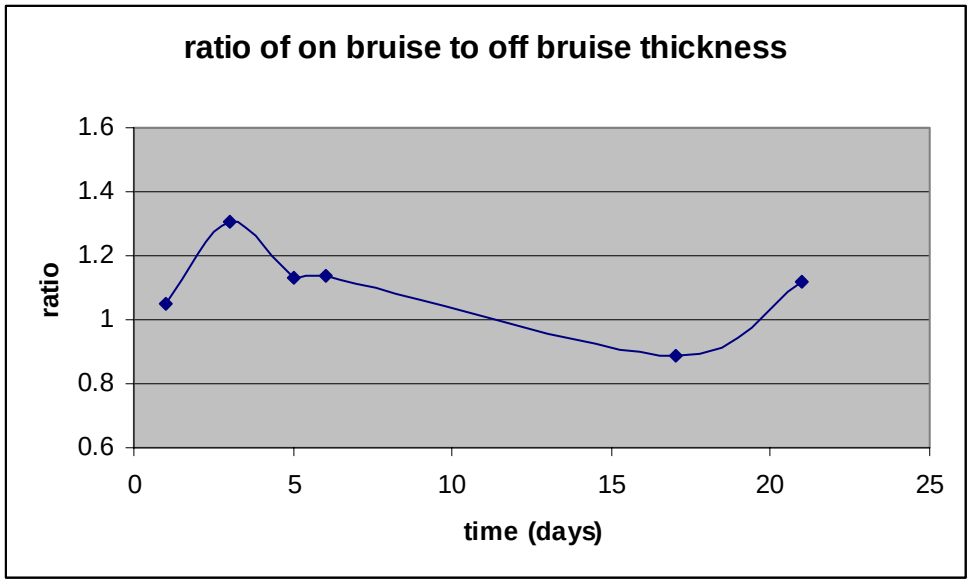


Figure 26: Ratio of On Bruise to Off Bruise Thickness

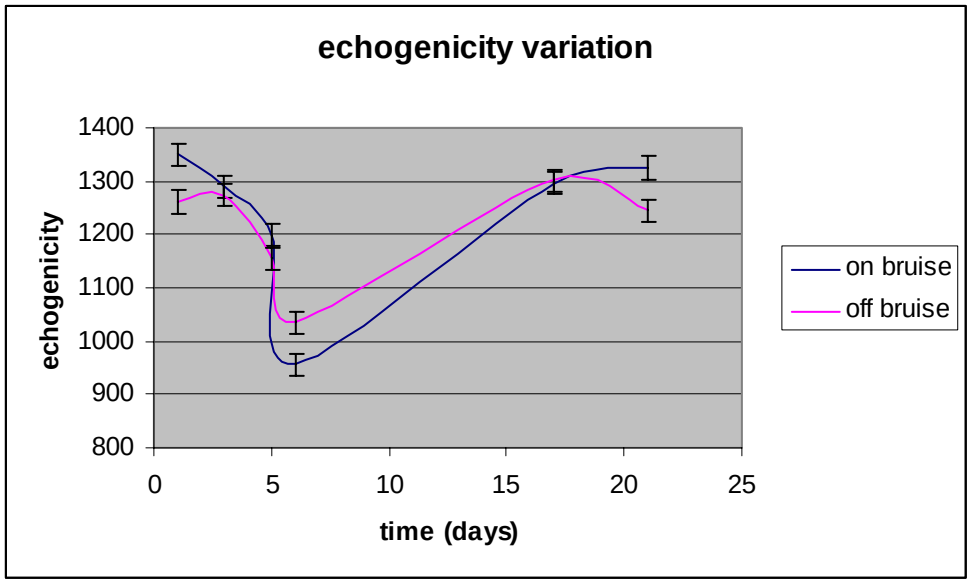


Figure 27: Echogenicity Variation

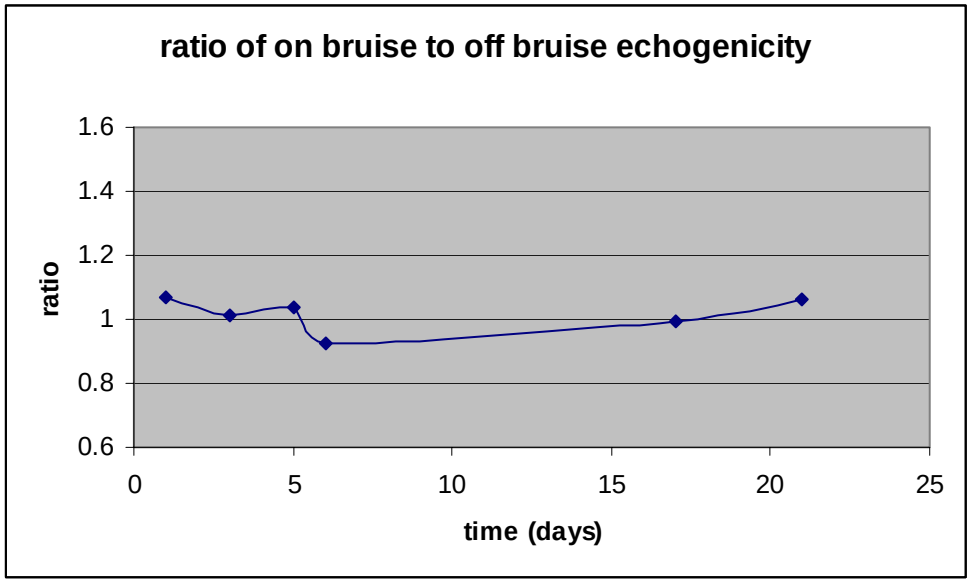


Figure 28: Ratio of On Bruise to Off Bruise Echogenicity

4.4.3 Subject B1103B

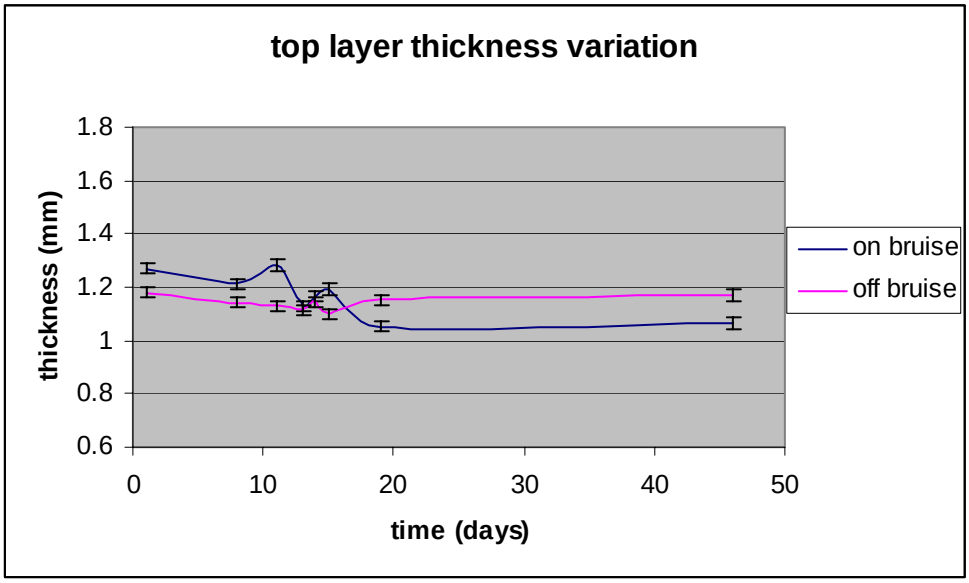


Figure 29: Top Layer Thickness Variation

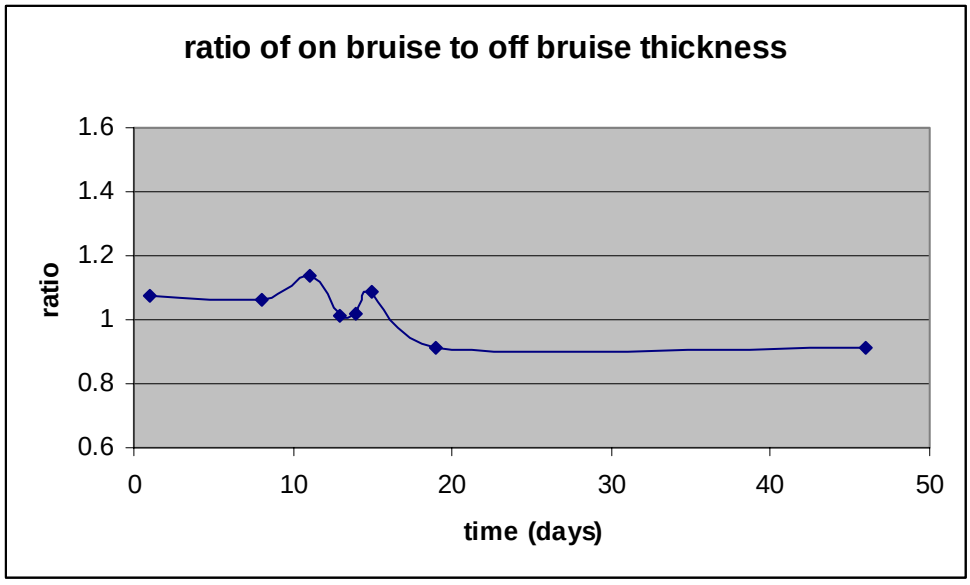


Figure 30: Ratio of On Bruise to Off Bruise Thickness

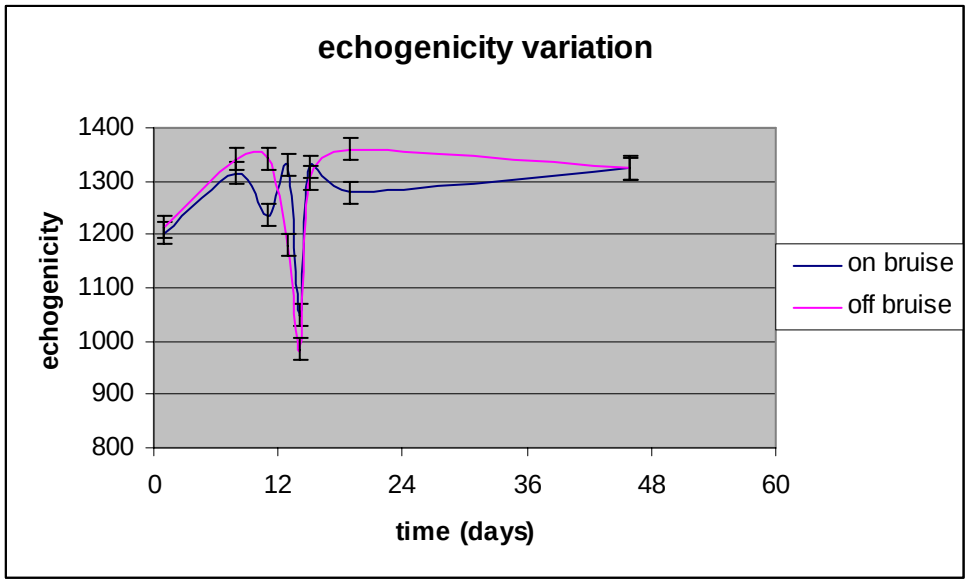


Figure 31: Echogenicity Variation

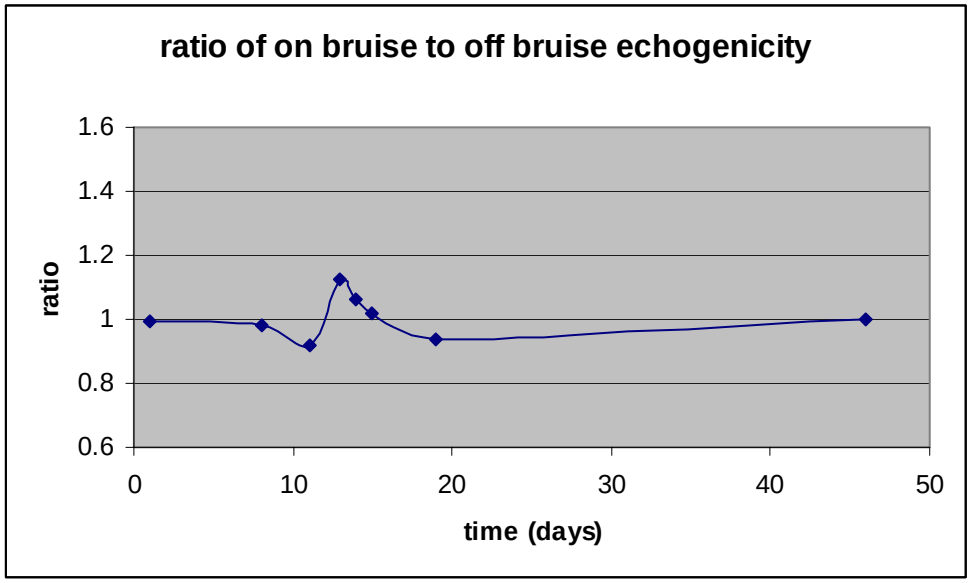


Figure 32: Ratio of On Bruise to Off Bruise Echogenicity

4.4.4 Subject B0929A

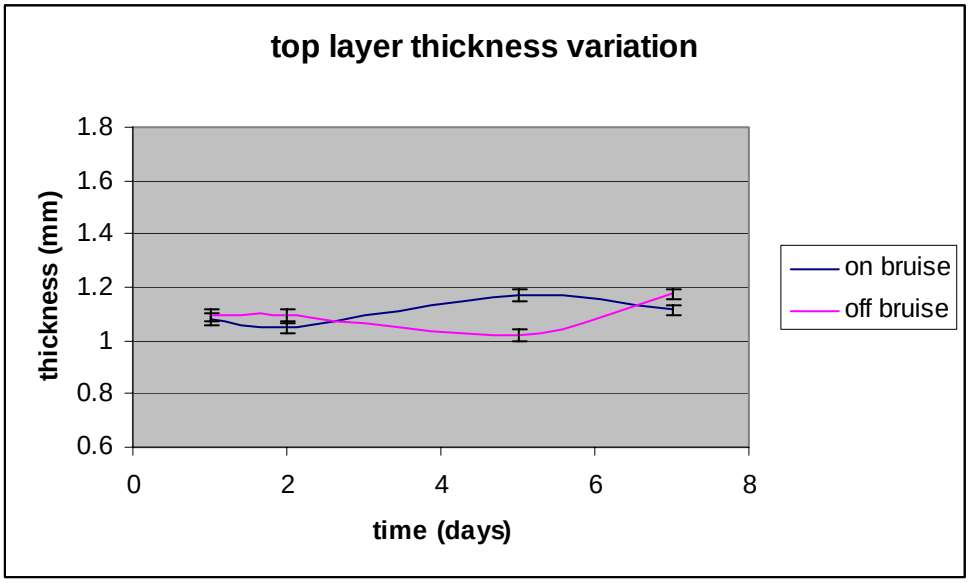


Figure 33: Top Layer Thickness Variation

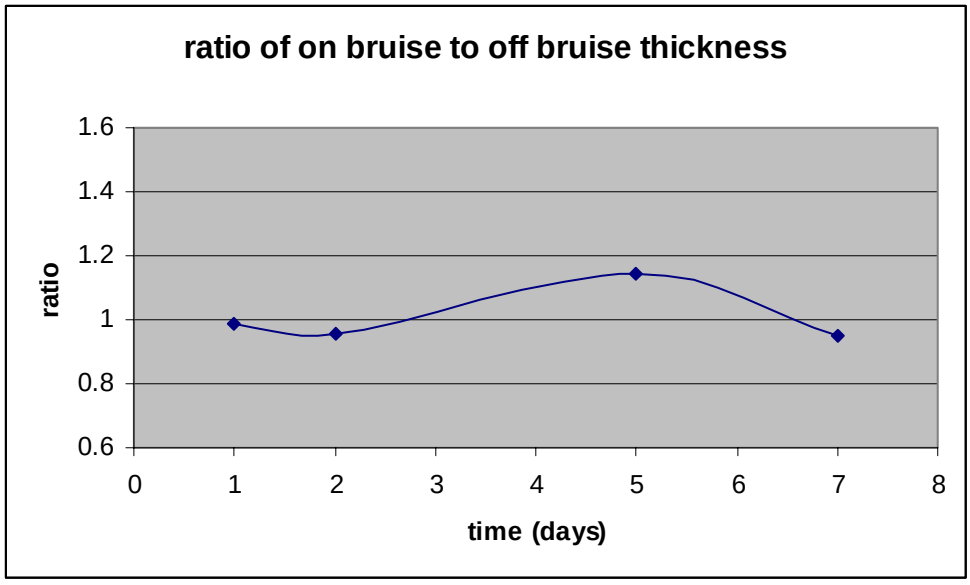


Figure 34: Ratio of On Bruise to Off Bruise Thickness

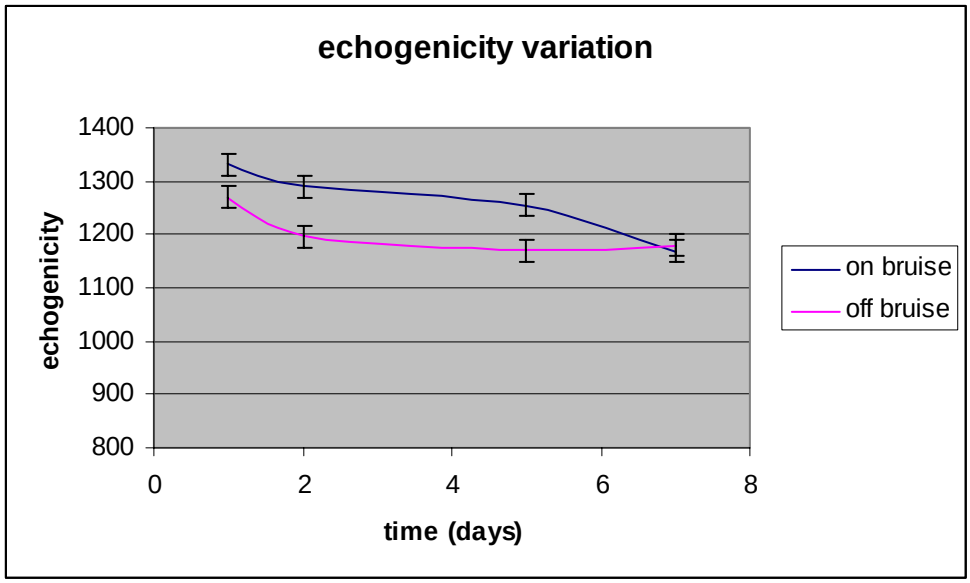


Figure 35: Echogenicity Variation

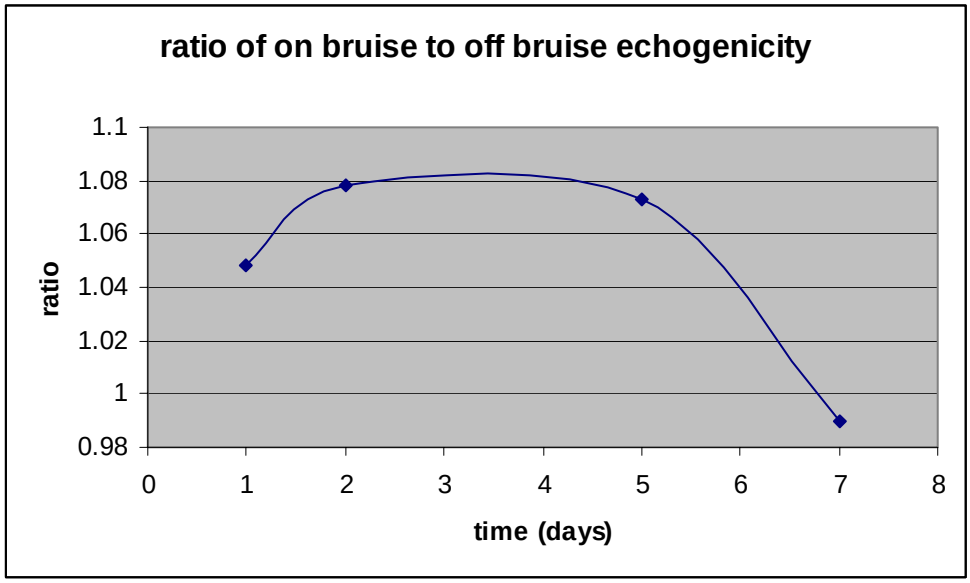


Figure 36: Ratio of On Bruise to Off Bruise Echogenicity

4.4.5 Subject B0930A

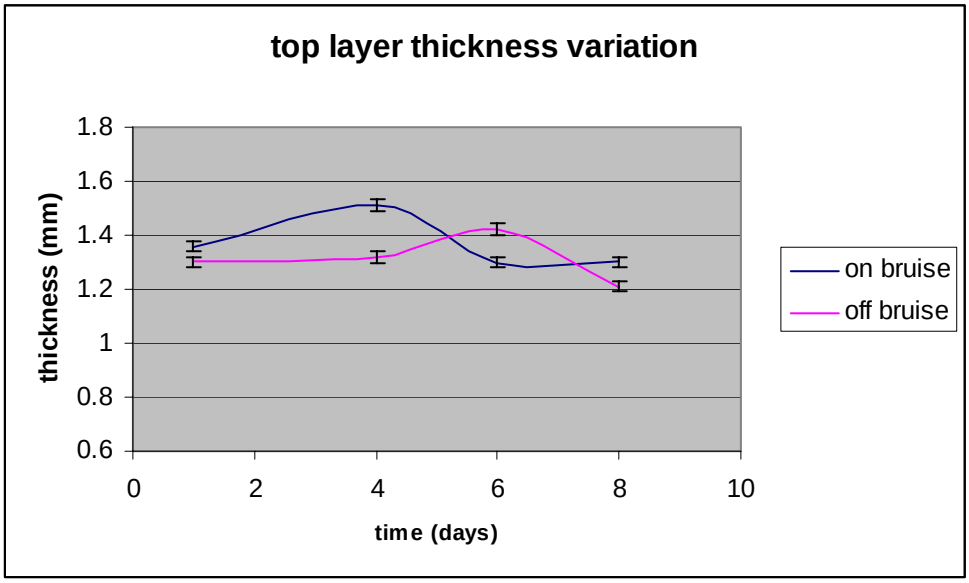


Figure 37: Top Layer Thickness Variation

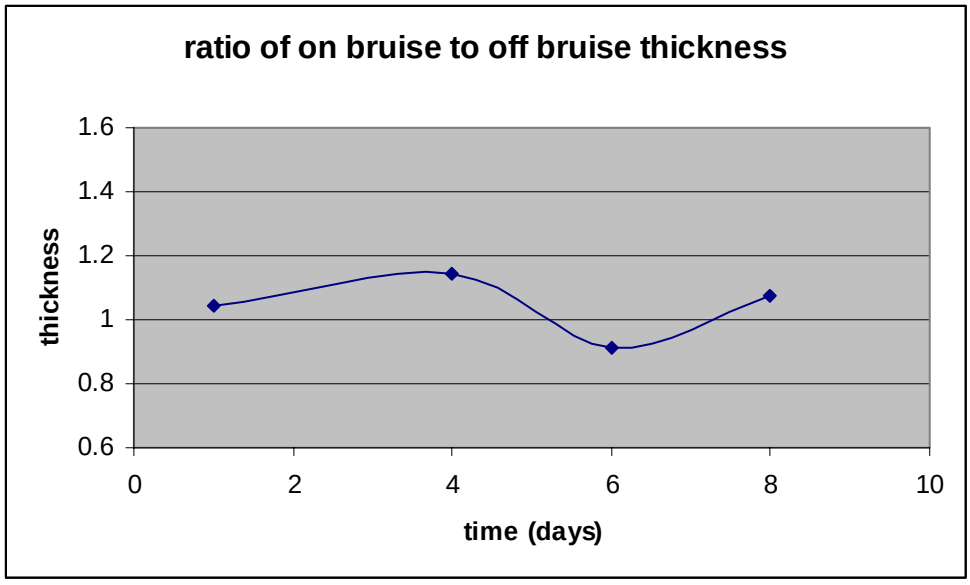


Figure 38: Ratio of On Bruise to Off Bruise Thickness

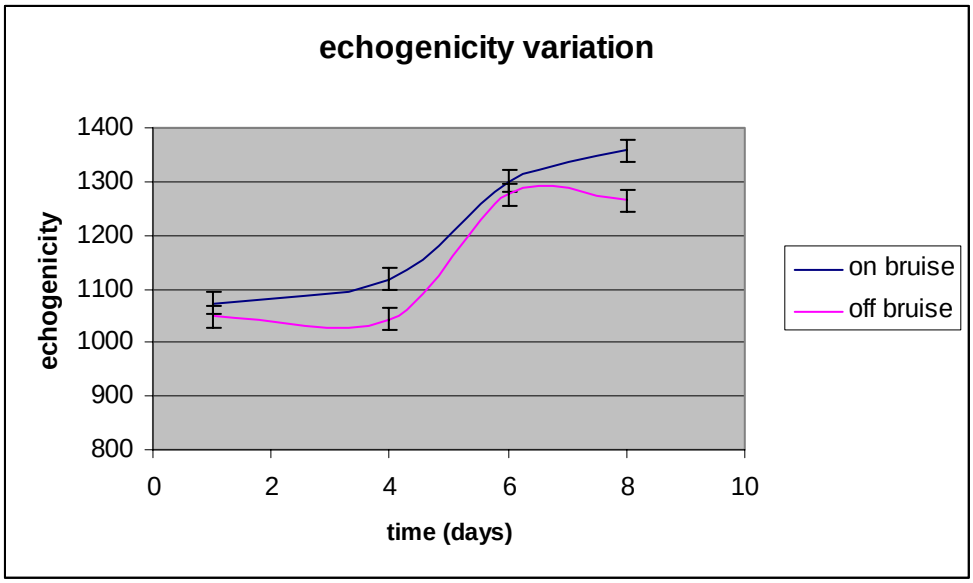


Figure 39: Echogenicity Variation

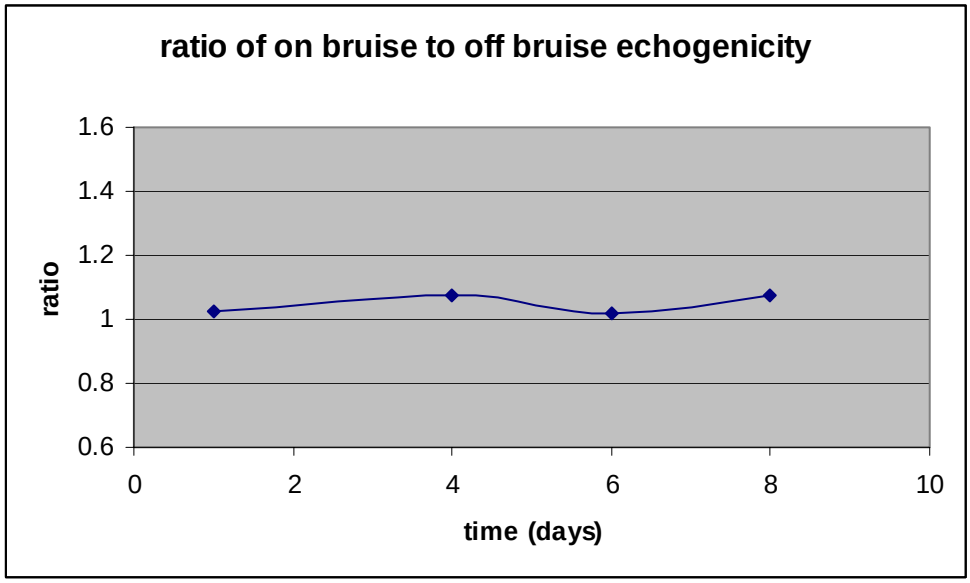


Figure 40: Ratio of On Bruise to Off Bruise Echogenicity

4.4.6 Subject B060120A

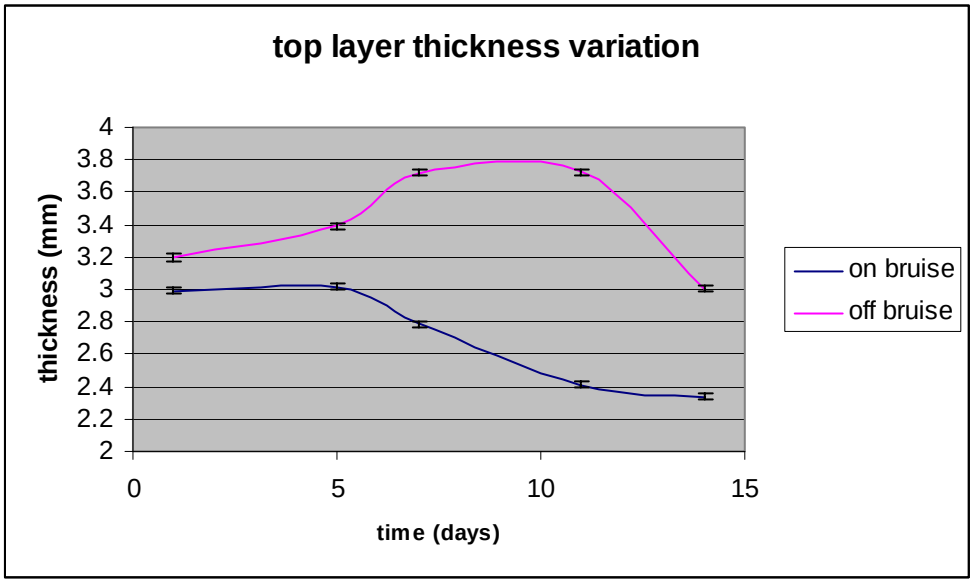


Figure 41: Top Layer Thickness Variation

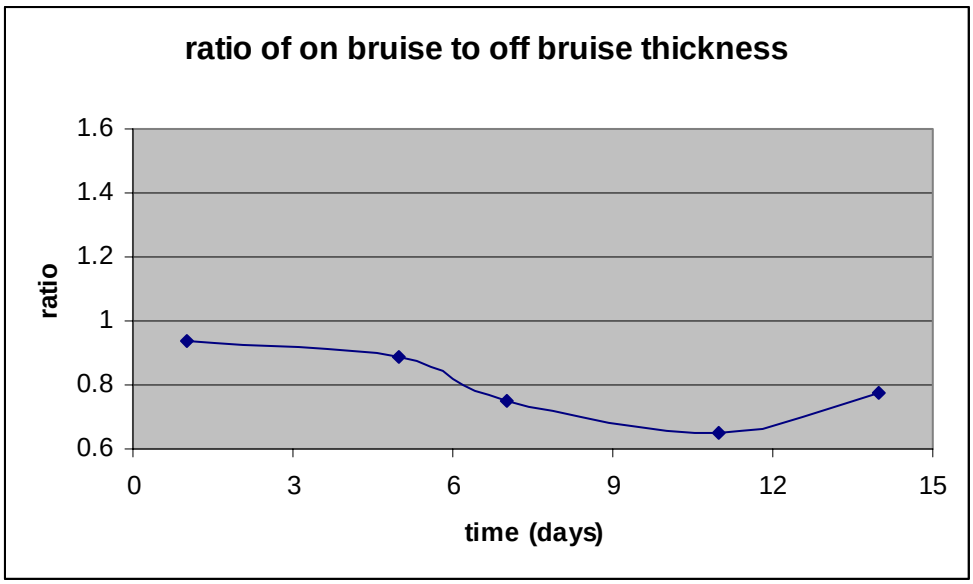


Figure 42: Ratio of On Bruise to Off Bruise Thickness

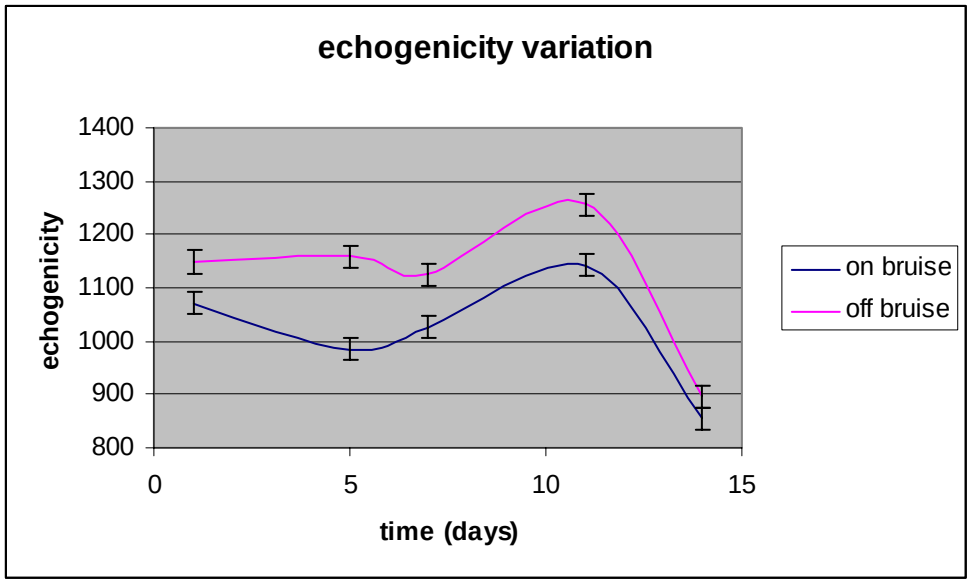


Figure 43: Echogenicity Variation

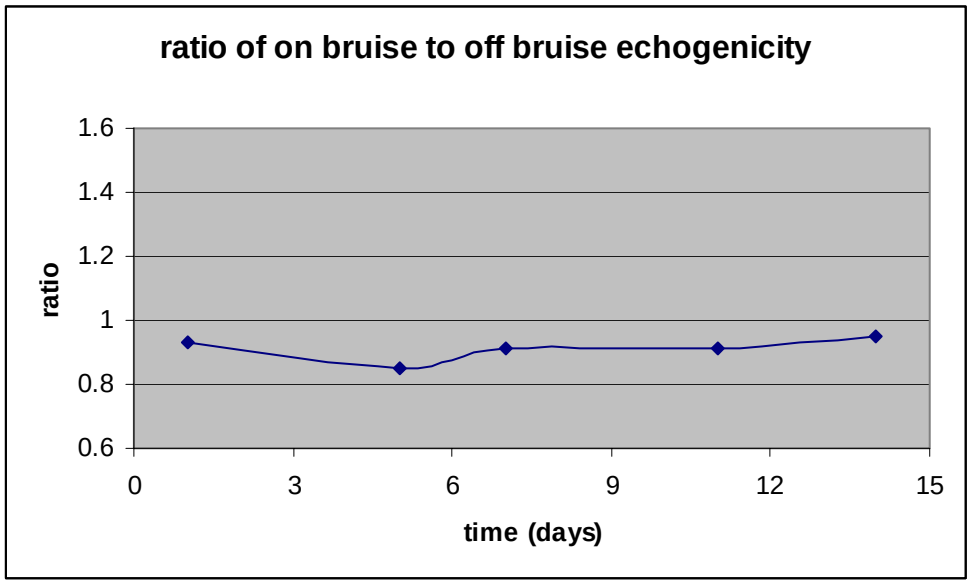


Figure 44: Ratio of On Bruise to Off Bruise Echogenicity

4.4.7 Subject B1028A

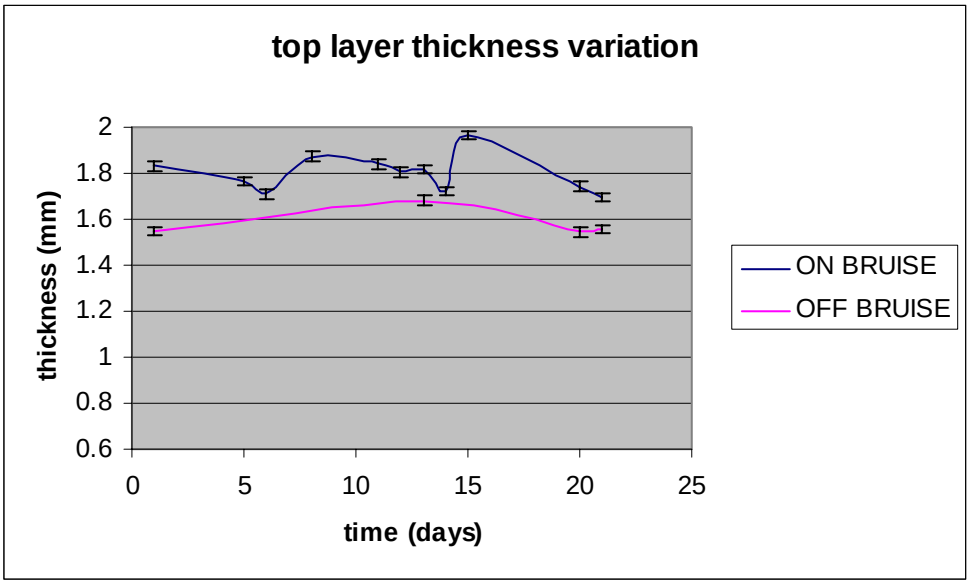


Figure 45: Top Layer Thickness Variation

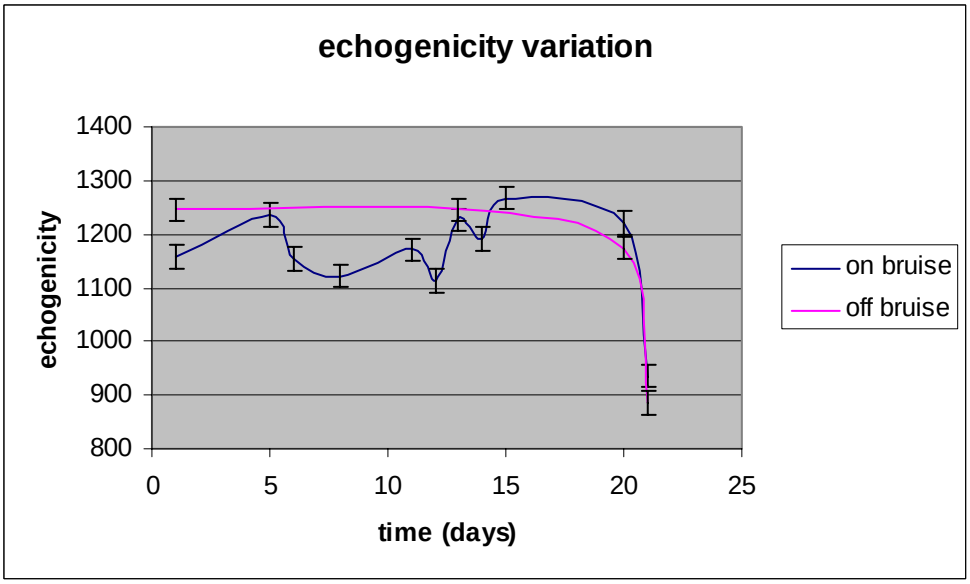


Figure 46: Echogenicity Variation

4.5 Snapshot Analysis

Results till now focused on quantifying the echogenicity and the top layer thickness values and contrasting those values for on-bruise and off-bruise sites. We saw the variation in the top layer thickness and the echogenicity for the on-bruise and the control sites over a period of time. This section will focus on the analysis of the B-scans on a particular day and try to elicit the qualitative changes between the B-scans of the bruised and the control sites.

The manifestation of an injury in the ultrasound images depend on a number of factors. The features of an injury might change depending on the age of the bruise, the gender of the patient, the age of the patient or the part of the body that is injured. The mechanism of injury and healing will be different for a blunt/impact bruise as compared to a punctured bruise. Keeping this in mind the changes which can be seen in the bruise image compared with the control site image are as shown below. The region of interest in each B-scan is marked with a yellow box.

4.5.1 Lower top-layer boundary becomes fuzzy

Figure 47 and Figure 48 shows the comparison of a control B-scan and an on-bruise B-scan. Figure 48 shows that the lower boundary of the top-layer of the skin becomes fuzzy or breaks down in the event of an injury. Notice that both the pictures have a clean epidermal boundary. It is only the lower boundary which loses its crispness due to the presence of a bruise.

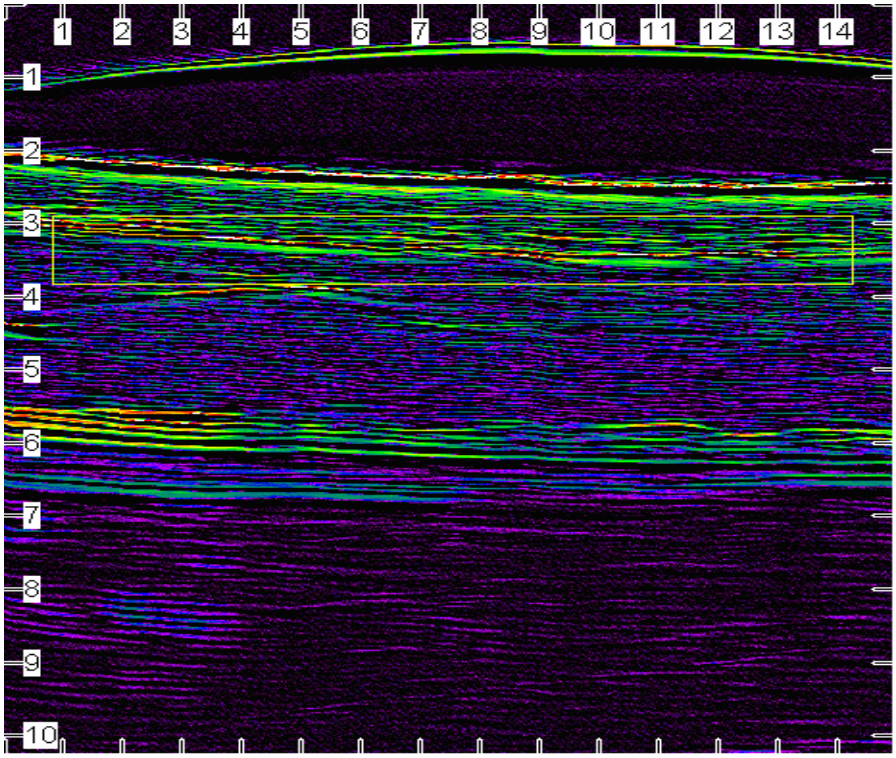


Figure 47: B-scan of Off Bruise Site

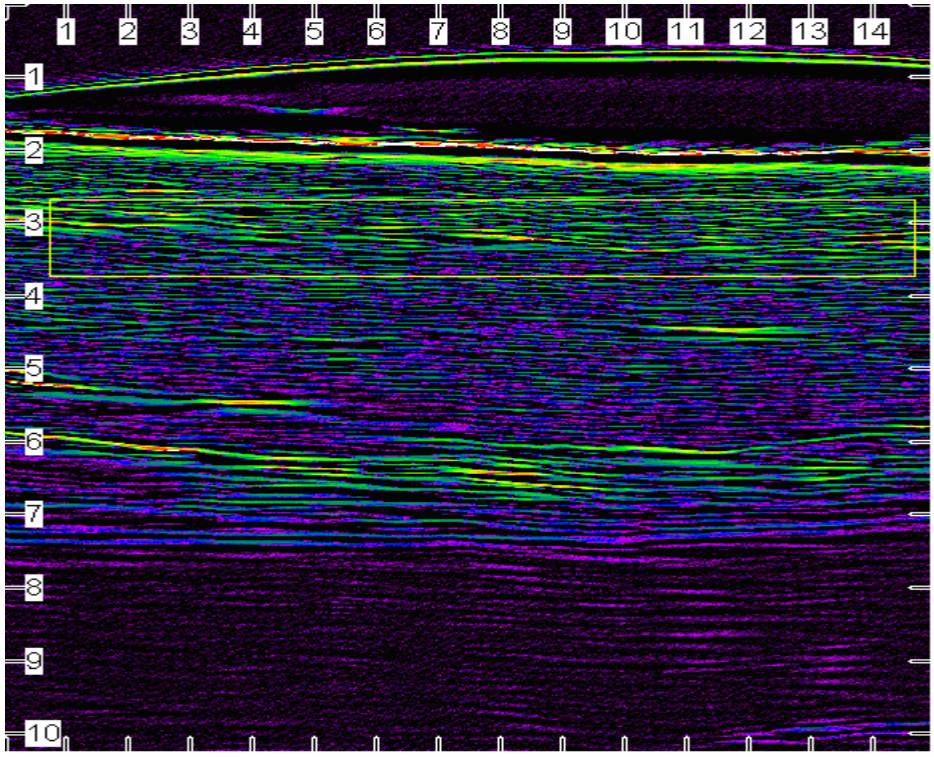


Figure 48: B-scan of On Bruise Site

4.5.2 Swelling in the bruise

The following two pictures show the difference between the B-scans of the bruise accompanied by swelling. Notice in Figure 49 that along with the breakdown of the lower top-layer boundary a clearly defined hypo-echoic region exists in the bottom of the on-bruise picture. This shows the presence of fluids in the bruised area manifested clearly in the ultrasound images. However, most of the bruised cases in this study were not accompanied with a swelling.

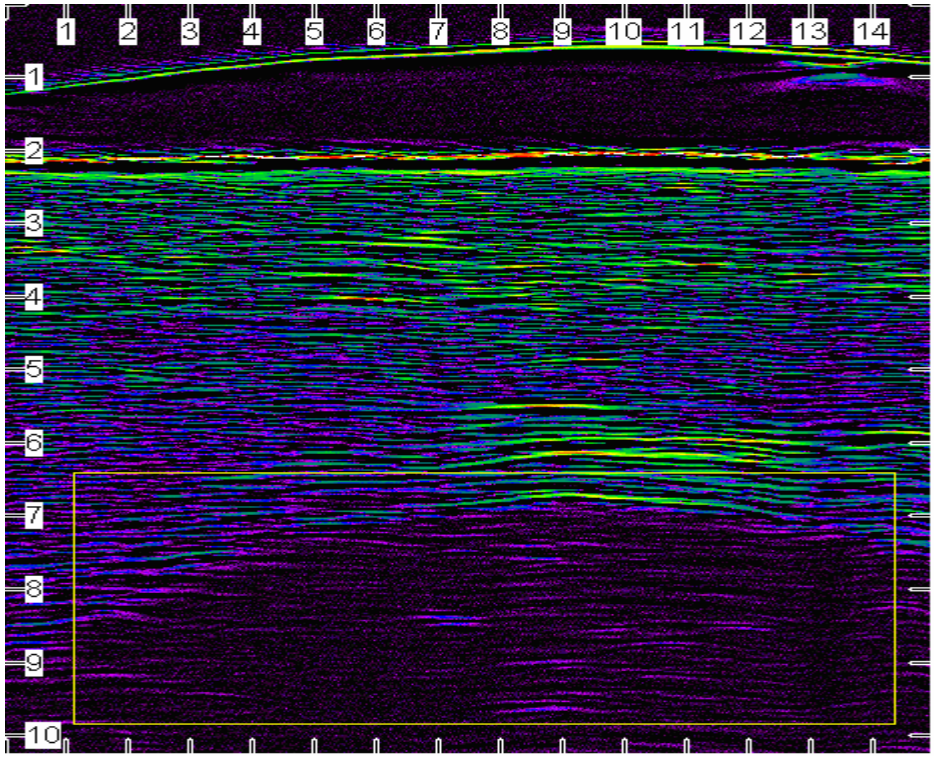


Figure 49: B-scan of On Bruise Site

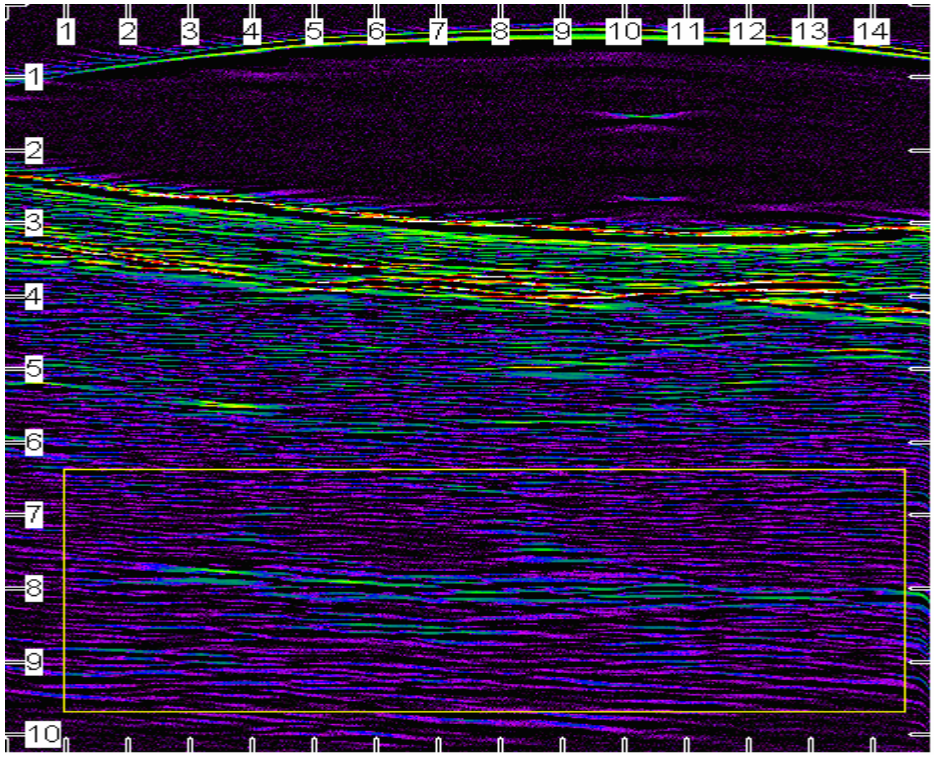


Figure 50: B-scan of Off Bruise Site

4.5.3 Change in top-layer thickness

In the event of an injury the top layer of the skin changes in thickness. In the previously shown results we have seen that the thickness of the top layer of the bruised skin is different from that of the non-bruise skin thickness. At this point, however, it is difficult to say whether there will be an increase or decrease in the thickness of the bruised site compared to the non-bruised or the contralateral site on a particular day. The thickness of the top layer in Figure 51 was found out to be 1.65 mm. The thickness of the top layer in Figure 52 was found out to be 1.35 mm. It should be noted that a change in the top layer thickness can only suggest the presence of an injury. It will be incorrect to say that the image with a lesser skin thickness is a bruise or vice versa. The presence of a bruise can be further confirmed by finding other visual indicators as mentioned in this section.

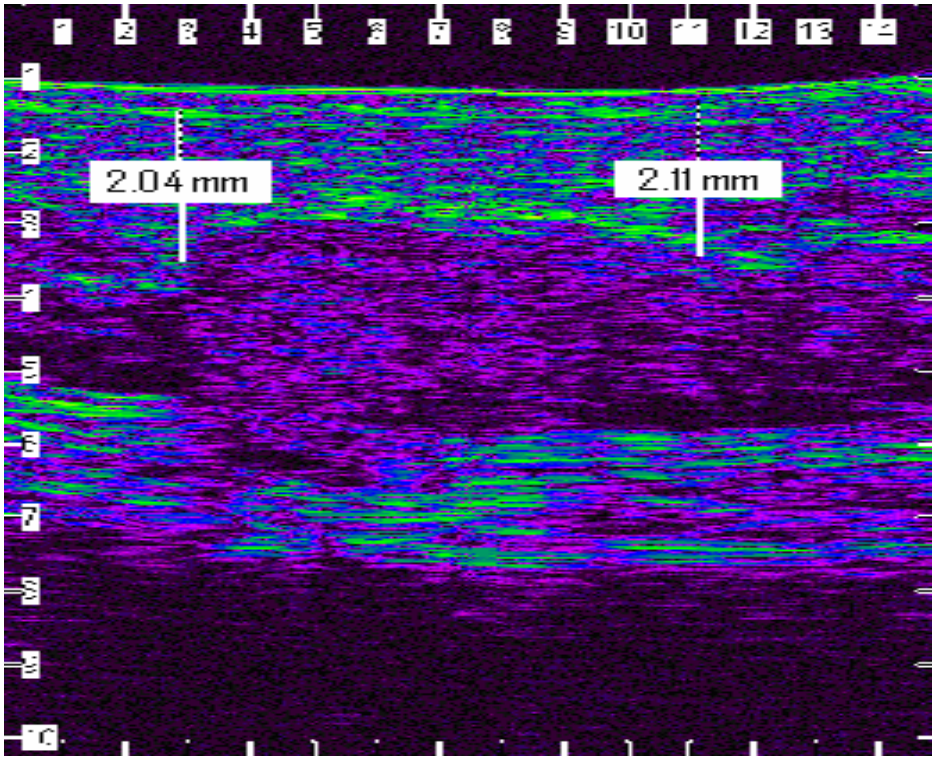


Figure 51: B-scan of Off Bruise Site

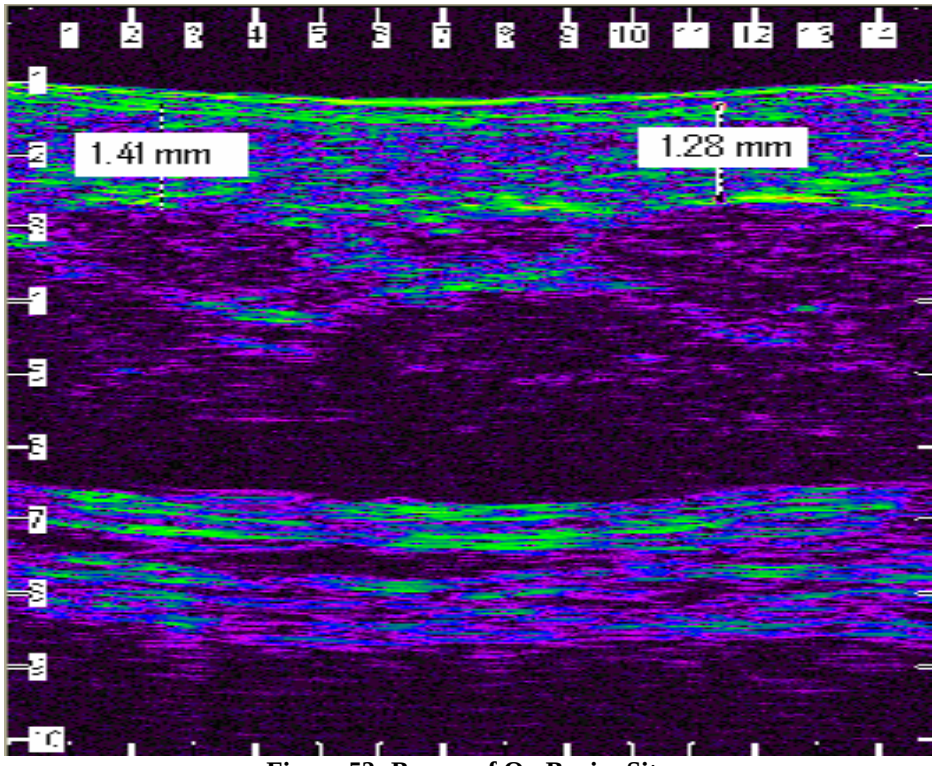


Figure 52: B-scan of On Bruise Site

4.5.4 Increased irregular echogenic regions

In some bruises there are irregular echogenic regions in the ultrasound images. This is especially true in case of bruises caused by concentrated forces like a needle stick. The bruise shown in Figure 53 was formed when blood was drawn from the subject's arm with a needle. Due to the concentrated nature of this force, irregular regions with changed echogenicity will be seen in the ultrasound images.

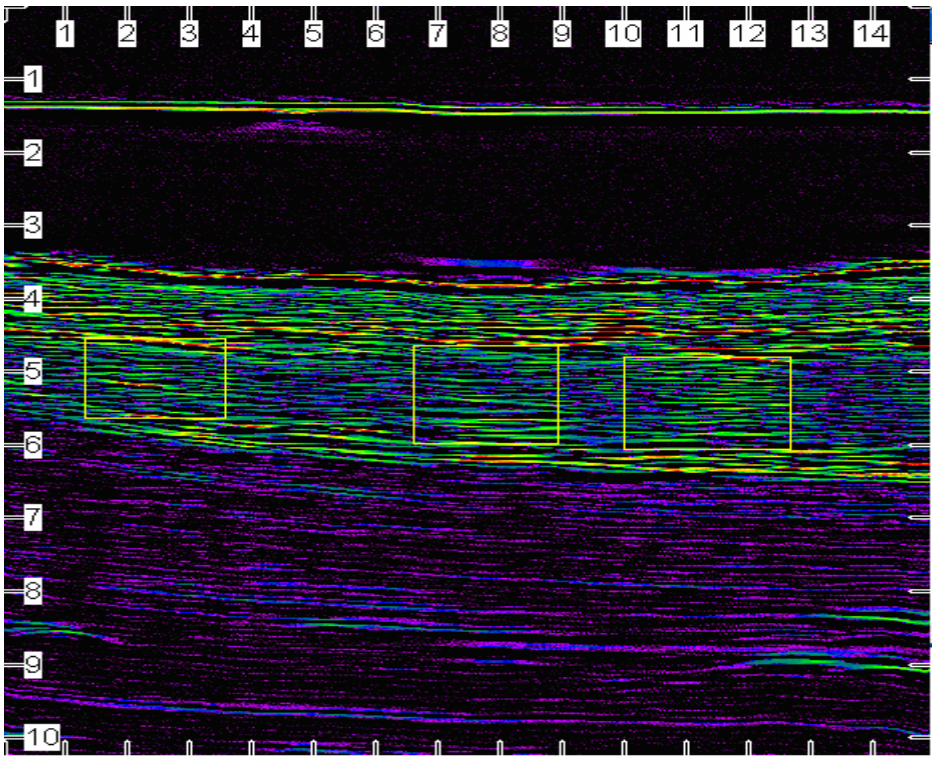


Figure 53: B-scan of On Bruise Site

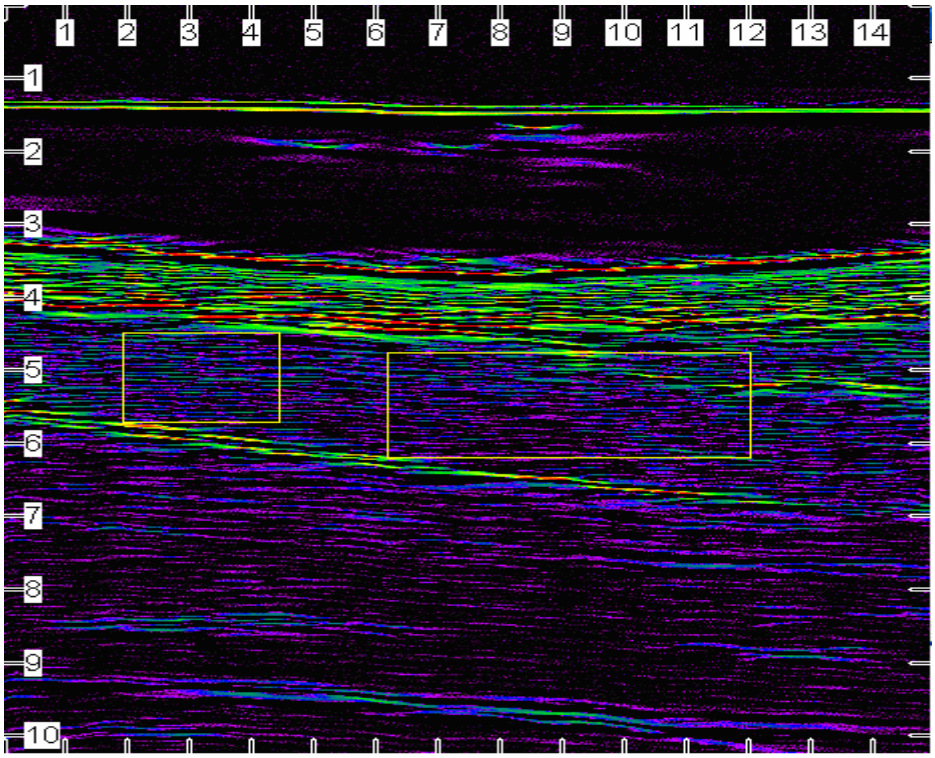


Figure 54: B-scan of Off Bruise Site

4.5.5 Change in Echogenicity

Change in echogenicity is one of the biggest clues in the determination of wounded tissue. Echogenicity has been quantified in the report earlier and its variation with time was seen. In most of the cases of blunt/impact bruises there will be a reduction in echogenicity of the bruise. However, in some cases an increase in echogenicity was also observed. The exact reason behind the increase in echogenicity in some cases versus a decrease in echogenicity may be caused by the expansion and contraction of the bruised region which occurs during the healing process. Figure 55 and Figure 56 have the echogenicity in the region of interest as 1210 and 1100 respectively.

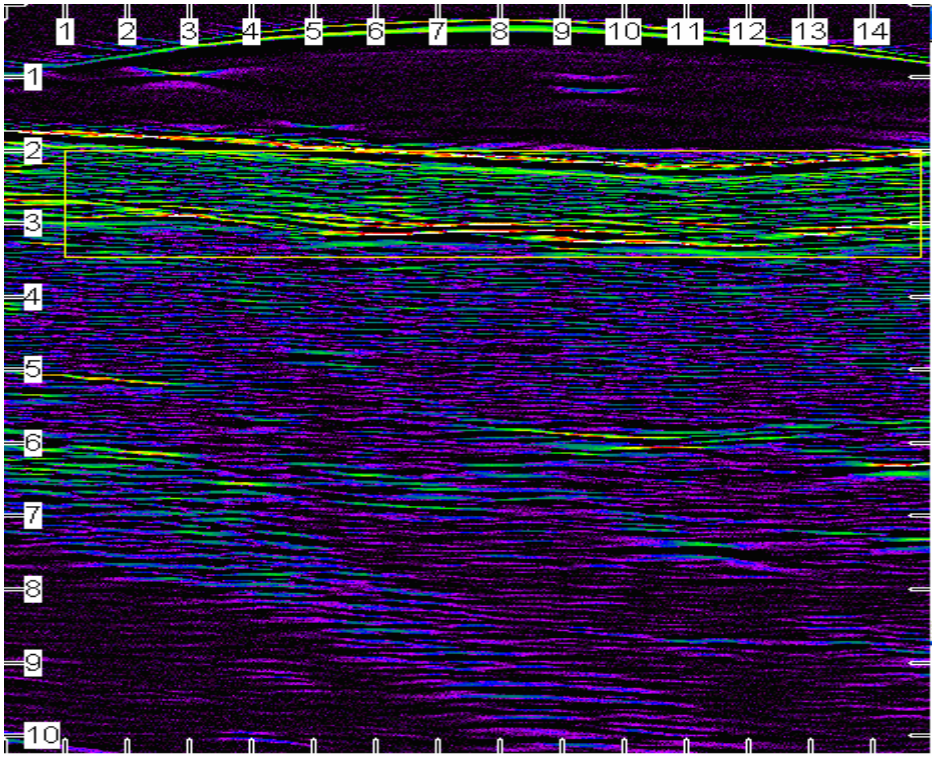


Figure 55: B-scan of Off Bruise Site

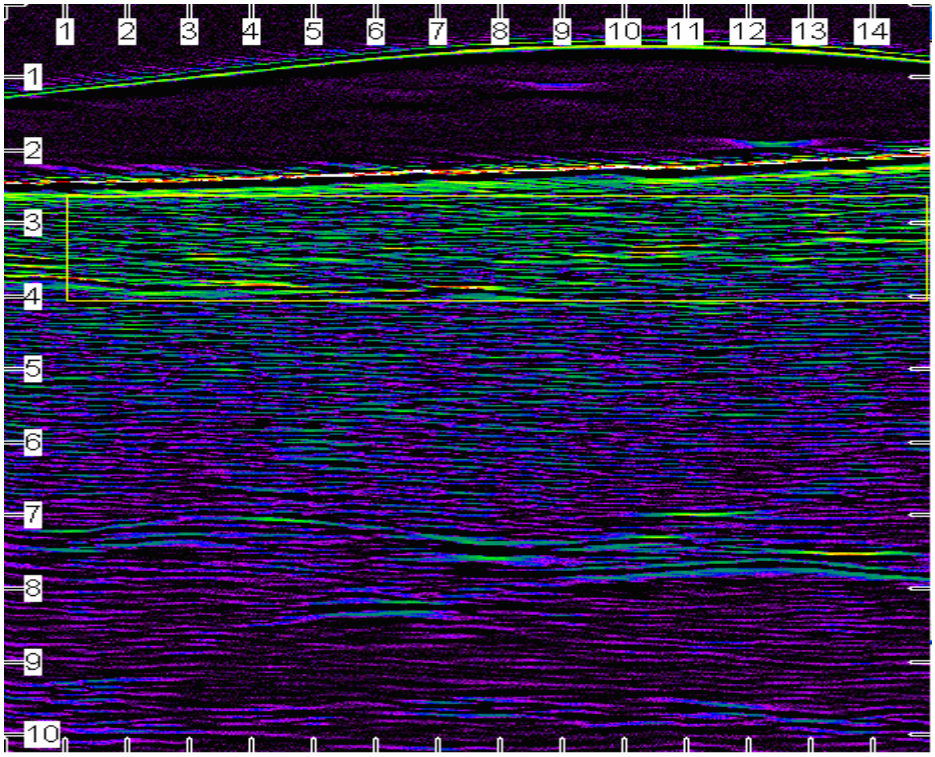


Figure 56: B-scan of On Bruise Site

CHAPTER 5

DISCUSSIONS AND CONCLUSIONS

5.1 Discussion of the Results

The data collection and analysis aims to find out the manifestations of an impact or a blunt force bruise in an ultrasound B-scan. These features are easily discernable for a laceration with a split in the epidermis or a late stage pressure ulcer. Figure 57 shows a laceration in which the loss of epidermis can be easily seen [2].

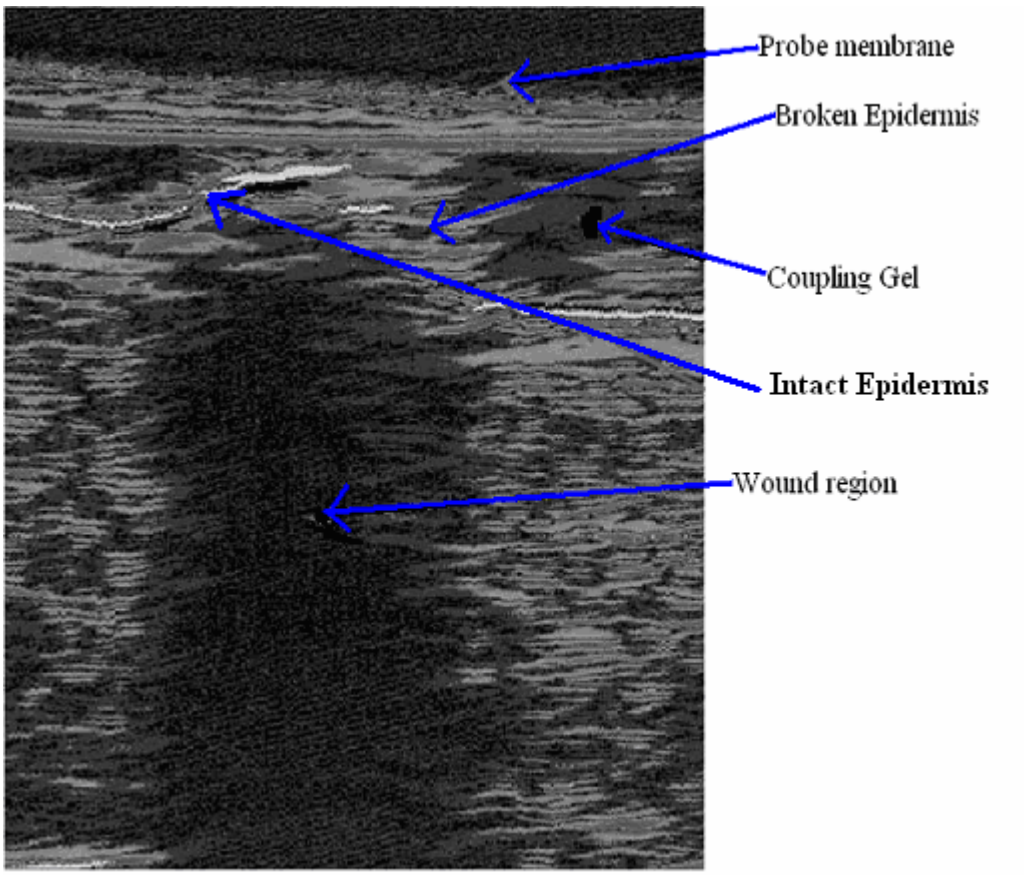


Figure 57: B-scan showing a Punctured Epidermis

Beneath the punctured epidermis a loss of tissue is manifested as a hypoechoic region. In Figure 58 is shown a pressure ulcer in which a similar hypoechoic region is manifested in the B-scan albeit at a different skin depth [26].

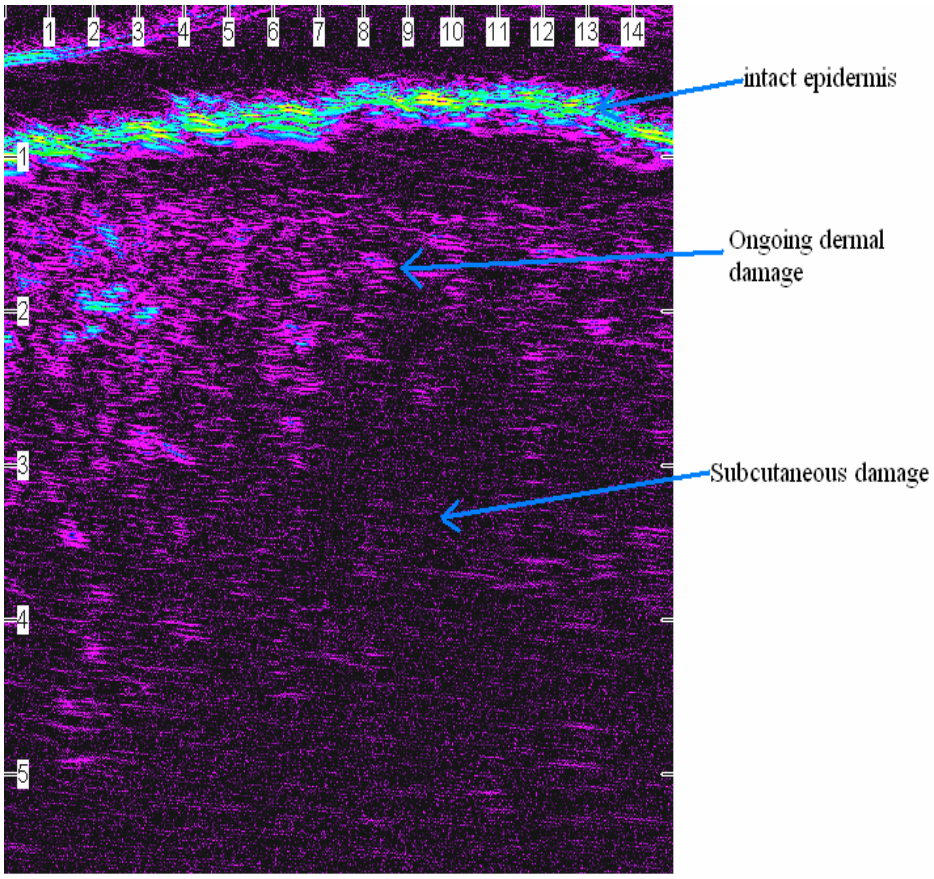


Figure 58: B-scan showing a Pressure Ulcer

This is because some pressure ulcers originate at bony prominences and then progress outward. The challenge in this research was to elicit such easily recognizable features for impact bruises where the epidermis is intact. However, such clear and quantifiable manifestations do not appear in the B-scans in a bruise caused by blunt force or impact.

This project began by characterizing the Longport EPISCAN to ensure that the best B-scan images were collected in terms of resolution, signal to noise ratio. Phantom

studies helped to simulate the measurement conditions and find out the best possible parameters. These chosen parameters when then tested by introducing bubbles and water bodies in the phantoms. The depth setting was chosen to be 10.2 mm because our subjects had bruises as opposed to deep tissue injury or pressure ulcers. Since most of the bruises manifest in the top layers of the skin that depth setting was considered appropriate. Experiments done by changing the orientation of the probe and pressure applied by the probe showed that the orientation of the probe did not significantly affect the B-scans. The EPISCAN has a resolution of 65 microns.

A comparison of the FFT and the RMS methods to measure echogenicity was done. In the FFT method the boundaries of the top layer is automatically detected. There is a characteristic trough in the echogenicity values at the epidermis and the lower boundary of the dermis is the point of maximum slope in the echogenicity variation. A comparison of echogenicity variations using the FFT and the RMS method was done to see which of them was better in the detection of the boundaries of the top layer. The COV for echogenicity measurements using the FFT method was 0.003 and the COV for echogenicity measurements using RMS method was 0.01. Since inter-operator reliability is a concern in our analysis, the method with the lesser COV is preferable.

FFT method for calculating the echogenicity also gives a flexibility to enhance certain frequency components and de-emphasize others. The variation in echogenicity along the depth of the scan was used to find out the boundaries of the top layer in the B-scans. Using the FFT method it was seen that the variation in echogenicity along the depth of the image is smooth and hence different layers in the B-scan can be found out easily. Using the RMS method it was seen that the echogenicity variation is abrupt all

along the depth of the B-scan. Finding out the actual boundaries of the top layer using the RMS method is therefore not very practical. Hence, the FFT method for calculating the echogenicity was used in all our measurements.

The top layer thickness and echogenicity data were used to visualize changes occurring over time. In the thickness and the echogenicity results, we notice a short term intraday variation and a longer term daily fluctuation. The fluctuation was found not to have any particular period associated with it. To get a sense of the time scale at which the variation in the skin thickness takes place the motor driving the transducer was stopped and a series of A-scans of one point on the body was taken. The EPISCAN takes about 512 measurements in a second. Figure 14 shows the variations in skin thickness within such a short time frame and the fluctuations are in the same order as those seen in the daily normal skin thickness. The peak to peak range of these fluctuations is more than 0.15 mm. The variation of skin thickness is much higher than the resolution of the device and also the SEM values. Therefore, these variations are significant. To make sure that the fluctuations seen in Figure 14 are not due to operator or machine error the same experiment was performed on a piece of plexiglass. This ensured that the results would only show the fluctuations due to operator technique or machine error. Figure 15 shows the fluctuations in the thickness of the plexiglass. The fluctuations have a peak to peak range of 0.04 mm which is lesser than the peak to peak range of the fluctuations for normal skin. This shows that the error introduced in the measurements due to operator or the machine is not significant. Therefore, the fluctuations seen in the normal or the bruised skin can be attributed to physiological reasons.

The normal skin thickness can change with time due to numerous physiological variables that affect it. These variations may arise from factors such as skin hydration content, blood pressure, or blood flow. Therefore, the analysis of the data should include some sort of normalization which will account for these short term and long term variations. In this study, this variation was normalized by taking the ratio of the on bruise and the off bruise data for top layer thickness and the echogenicity.

The variations in the data for thickness and echogenicity are numerically significant because the variations are of much higher magnitude compared to the SEM or the machine resolution values. However, the thickness and echogenicity data does not show any discernable and specific trend over time. One of the shortcomings of the data is that the data collection was stopped the day the bruise appeared to have resolved visually. A change in the collection protocol has been implemented which tracks a bruise a week after the visual resolution occurs.

The results also question the validity of research on skin thickness changes that have been observed as an indicator of several pathological conditions or as an indicator of the efficacy of certain drugs. From this research, there is reason to believe that the skin thickness can change by 0.1 – 0.2 mm depending on the time of the day the scans are taken. Therefore, a conclusive result based on skin thickness variation or change should consider the above variation.

Even though there is no discernable trend in the thickness or the echogenicity variations over time there are several important points worth considering. The time for healing of the bruises differ from case to case. The time to heal depends on factors like the extent of injury, the part of the body that is bruised, the gender and the health of the

subject. It should be noted that the values of thickness or the echogenicity of the bruised area are not consistently higher or lower than that of the off bruise or control site. This might be due to the contraction motion in the healing process. The range of the ratio of on bruise to off bruise thickness and echogenicity is between of 0.8 and 1.4 for thickness variation for most cases. The range of the ratios for echogenicity variation is between 0.6 and 1.2 for most cases. These range values gives a good guideline about the magnitude of variations to expect in case of impact bruises.

The other approach was to compare on bruise B-scans with off bruise B-scans to find the differing structural features in them. At this point it must be mentioned that it is only a relative comparison between these scans that can help us identify the bruised region. A standalone scan by itself cannot be used conclusively to determine a bruised region. There are several subcategories which can help us look for clues in the ultrasound scans as listed below:

1. Change in the top layer thickness
2. Change in the top layer echogenicity
3. The lower boundary of the top layer becomes indistinct or fuzzy
4. The presence of a hypoechoic region corresponding to fluid which causes swelling below the top layer
5. The presence of irregular hyperechoic regions corresponding to blood clots along the fascia below the top layer
6. The presence of a hypoechoic strata just below the epidermis which is indicative of superficial bruises within the top layer

To narrow down the criteria a thorough assessment of the age of the bruise, the cause of the injury and the part of the body injured must be made. For example, if bruise palpation feels like swelling or edema hypoechoic region in the dermal and the subcutaneous regions can be expected. From the analysis of many sets of data it was seen that an indistinct lower boundary of the dermis is one of the most consistent manifestations of a bruise. A change in echogenicity is also occurs often in a bruise although it is difficult to say whether it will be higher or lower than the off bruise sites. The snapshot analysis gives us clues which can further be quantified using algorithms which are similar to those used in pattern recognition or face recognition.

5.2 Conclusions

1. The thickness and the echogenicity of the top layer of the skin fluctuate even in normal physiological conditions. This fluctuation needs to be considered when conducting research on tissue properties.
2. The thickness and echogenicity of the bruised region also changes but these values can be greater or lesser than the normal region on a given day. In normal skin these changes are cause by physiological variables. Further investigation into how these variables change with bruise healing may allow prediction of the relation between these values.
3. The range of the ratio of on bruise to off bruise thickness was found to be between 0.8-1.4.
4. The range of ratio of on bruise to off bruise echogenicity was found to be between 0.6 and 1.2.

5. There are several qualitative features which can detect bruise images and can be used to distinguish them from control site images. Usually, more than one of these features can be found in the bruised B-scans for additional confirmation.

5.3 Future Work

Future work involves data collection with the new and improved protocol which will ensure clean ultrasound B-scans captured on a daily time frame. The bruises will be tracked at least a week after visual resolution is achieved to accommodate for any non-visual indicators which may still lie beneath the surface. The method for determining the echogenicity may be further refined by taking into consideration the attenuation of the signal along the length of the scan and normalizing all the scans based on a reference value. To regulate the frequency of scans and the physiological conditions of the tissue, working with porcine bruises may help eliminate the problems associated with the data collection. The phenomenon then observed will be more reliable with more data points. Since the final aim of this project is to develop a handheld and affordable device which can be used clinically to determine bruises and deep tissue injury, efforts should be directed towards developing a smaller ultrasound unit with appropriate detection algorithms. The features pointed out in the snapshot analysis can be further quantified using artificial intelligence algorithms for pattern recognition or face recognition.

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